SELENIUM DERIVATIVES OF BICYCLIC COMPOUNDS AS POTENTIAL CANCER THERAPEUTIC AGENTS

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I. Introduction

It has been estimated that more than 250,000 people in the United States will die this year of cancer. That is, approximately 700 Americans will die of cancer every day. By means of improved and earlier treatment, the ratio of one person saved out of four treated has now been raised to one saved out of every three treated. Because of the extent and severity of this disease, cancer research has become a separate field of study and has expanded until it now incorporates techniques from almost every basic science known today.

Cancer research has as its object the understanding, prevention and cure of cancer. Cancerous cells, however, are very similar to normal cells in many respects. Methods of treatment, which must differentiate between these two cell types, have thus been complicated by these cellular similarities. These difficulties, along with many others, have prevented investigators from completely achieving the above-mentioned goals of cancer research.

As in any other difficult problem, when all routine procedures fail, the path taken is a broader and more intensive scientific investigation. In this extension of the scope and intensity of research, the oncologist has now been forced to change from his study of organs and tissues to the components of these same units, extending his examinations down even to molecular proportions. At this level, researchers can now use the principles of physics and chemistry as a

foundation upon which sound correlations and conclusions can be based. At the molecular level, studies of the normal conditions which surround the living cell have led to a better understanding of what happens in the process of normal cell growth. These studies have demonstrated certain differences between the normal cell and the tumor cell.

The methods of treatment of cancer, available to the physician today, may be divided into two broad catagories. They are: first, the removal of neoplastic areas by surgery or radiation and second, the chemotherapeutic approach. The former, although more widely used than the latter, has several innate disadvantages. The most important of these is the inability of such methods to remove the important widespread neoplasms. Surgery and radiation procedures, by their very nature, are focal treatments. Success here depends upon the complete removal or destruction of a localized area. The high degree of success in the treatment of skin cancer is a result of its early recognition and accessibility. Here the problem is greatly reduced and cures are obtained in better than ninety per cent of such cases.

In the process of growth, the malignant tumors are seen to possess certain common characteristics. Among these is the property of invasiveness. That is, in the spreading growth of the tumor, it invades or grows through its neighboring tissues. In this uncontrolled expansion, the tumor grows at the expense of the neighboring tissue.

Another important characteristic of malignant tumors is that of metastatic growth. Such tumor growths are the result of the spread

of a primary tumor, via the blood and lymph vessels, to distant parts of the body.

From these two properties, it readily can be seen that a localized or focal treatment of cancer will leave behind the outgrowing segments of a tumor. The visible tumor may thus be removed, but the invisible extensions of the tumor are left to take their toll of lives. Once these outgrowths of the tumor become established, the use of radiation or surgery becomes a hopeless operation. Some of these secondary growths may be removed but it is usually only a matter of time before the tumor becomes too wide-spread to be treated.

From the above, the need for a treatment which can seek out all parts of a tumor, including its extensions, is readily apparent.

The answer to these problems is an agent which assumes a form suitable to the environment in which it must act.

The chemotherapeutic method offers such an agent. Here a drug is released into the circulatory system where it reaches out into the entire body. In such a treatment, every tumor cell will be reached regardless of its size or location in the body. Here indeed, the agent can seek out the tumor and exert its influence.

II. HISTORICAL

A. CANCER CHEMOTHERAPY

1. Early Chemotherapy.—In the first days of the treatment of cancer there was no knowledge of the manner in which neoplastic cells grow and multiply. There was, however, a recognition of a need to remove the cancerous tissue. Therefore, the agents which were used were those which would destroy the cancerous growth. The only available chemical agents which would achieve such an effect were the destructive poisons and caustics. These were applied in the hope of annihilating the neoplasm and replacing it by healthy scar tissue. However, while some cancers were cured by treatment with caustics and poisons, the terrible burns which resulted led to the abandonment of this form of therapy.

The choice of agents to be used was mainly based on empirical findings. This was not a matter of choice, but one of necessity. For, in order to have a rational approach to a problem, one must have a method for evaluating the treatment used. In the earliest times, such methods were unavailable. The only criteria, upon which the value of a drug could be based, were those of the general well-being of the patient, palpation and gross examination of the area treated.

The use of arsenic in the form of ointments containing arsenic trisulfide has been mentioned in the writings of Hippocrates, who lived from 460 to 377 B.C. (17). Arsenic trisulfide and red arsenic

disulfide were reported in the writings of Dioscorides and Pliny in the first century A.D.

During the Middle Ages, compounds of arsenic were used only externally due to the drastic toxic results shown in too many cases. Then, with the appearance of syphilis in Europe toward the end of the fifteenth century, arsenicals again became prominent. At that time a solution of potassium arsenite, known as Fowler's Solution, was the most widely used form of arsenic.

The problem of determining the properties of neoplastic tissue has been studied through the ages. It was not, however, until the seventeenth century when Athanasius Kircher first applied the microscope to the systematic study of diseases, that a feasible method was available. But, the importance of such a method was not accepted until the nineteenth century when R. Virchow applied the cell theory to pathology, demonstrating that pathological processes can be traced back to cellular events. With the development of the microscope and staining techniques, the possibility of studying the effects of chemical agents on the components of cells was realized.

Another important period in time is that during which the chemical and dye industries developed. During this period many coal tar workers were exposed to benzene fumes. Investigating the physiological effects of benzene, Koranyi (20h) discovered that benzene causes a reduction of the blood forming tissues of the body. The excess number of white blood cells in the leukemic patient, seemed a good target for this new-found effect of benzene. Koranyi studied the effect of

benzene on leucocytic counts and found a greater and more persistent decrease than with arsenic. Benzene was never seen to return the leucocytic count to normal, as has been shown in some later cases of x-ray treatment.

The toxic effects of benzene, which may be fatal, as well as the danger of inducing aplastic anemia (78) contraindicates its use.

One of the earliest uses of bacterial metabolites was for the treatment of cancer. Favorable influences upon the course of cancer were noted after an attack of erysipelas. Subsequently, upon experimental infection of patients with erysipelas, as well as other infections, some favorable results in cancer patients were observed. However, many statements in the literature neglected to describe the failures of such treatment and over-emphasized the beneficial effects (180). W. B. Coley reported a more easily reproducible method, involving fewer dangers than the erysipelas infection, in the form of injections of a toxin mixture prepared from Bacillus erysipelatos and Bacillus prodigiosus called "Coley's fluid" or "Coley's toxins" (41). Greenstein (82), after reviewing the data on the effects of this agent, has stated that although favorable reports are found, the lack of a standardization of the method renders an evaluation of such findings rather difficult.

More recently, Shear (196), working on bacterial cultures isolated in pure form, the material responsible for the hemorrhage and necrosis produced in cancer tissue and frankly pointed out the deficiencies in such treatments. Experimentally, doses tolerated in a

cancerous animal were lethal to normal animals.

2. Modern Chemotherapy.—Although one cannot choose a definite date when cancer chemotherapy passed into a modern era, it is generally agreed (70) that those discoveries made since the beginning of World War II include most of the important advances. The age of modern chemotherapy has been characterized by a rational approach founded on a thorough investigation of basic cell processes. From an understanding of these vital cellular reactions, the chemist and biochemist have to some extent been able to determine which substances act as intermediates in a particular biochemical reaction.

By slight modifications of biochemical intermediates, it has been possible, therefore, to block certain necessary cellular reactions. The theory here is that the cell cannot distinguish the modified from the natural intermediates. Hence, both are incorporated into the particular biochemical process. If then the cell cannot metabolize the modified molecule, the metabolic process will be blocked with perhaps fatal results to the cell. As defined by Greenstein (82), the chemotherapeutic agent should possess a specificity for the tumor cell. Hence, it is hoped that the blocking action will take place only in the cancer cell. The increased metabolic rate of the cancer cell may aid this theory in that the cancer cell will, with its stepped up metabolism, incorporate more of the altered molecules than the normal cell.

On the other hand, the chemotherapist has not been limited to those agents which enter into well understood biochemical processes.

The chemotherapy screening programs have been open to all types of compounds.

B. AGENTS USED IN CANCER CHEMOTHERAPY

1. Polyfunctional Alkylating Agents.—The agents which make up this class possess only a slight specificity of action. They derive their name from the process by which they affect biological intermediates, that is by alkylation.

The historical development of the alkylating agents began with sulfur mustard, or bis(2-chloroethyl)sulfide, I. This compound was

$$ClCH_2CH_2SCH_2CH_2Cl$$

I

synthesized in 1854 by the German chemist, Richie. Its biological properties were investigated by Victor Meyer in 1886, who noted its strong vesicant action along with its necrotizing effect on the skin, respiratory tract, and eyes. The Germans recognized the toxic possibilities of this substance and developed its use as a war gas with much success during World War I.

It was not until 1919 that the depression of the hemopoietic system was noted (127, 128). Adair and Bagg in 1931 (1) made an attempt to use this compound as a cancer chemotherapeutic agent, but were forced to abandon its use due to difficulties in handling the agent.

The discovery of the alkyl nitrogen mustards (215), gave to the oncologist a much more easily handled agent. These compounds are of the general type, II, R = alkyl. The clinical studies with these

$$R\text{-}N(CH_2CH_2Cl)_2$$

II

compounds in the treatment of neoplastic diseases was initiated at Yale University in 1943 under Goodman et al. In 1946 this same group (80) reported the results of their earlier experiments. These appeared at about the same time as those of Jacobson et al. (117) of the University of Chicago. Since then, numerous publications have confirmed these results.

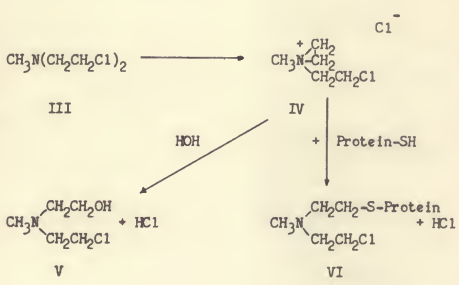
Destruction of lymphoid tissue and depression of the hemopoietic function are the effects produced in the normal organism when therapeutic doses of nitrogen mustards are given. These toxic effects on normal tissues accompany the therapeutic action of nitrogen mustards in the treatment of certain neoplastic diseases (70). Despite this limitation, nitrogen mustards are of great importance and are still the subject of much research.

In the past decade, Haddow's group at the Chester Beatty Research Institute have been active in studies on nitrogen mustard type compounds. Of particular interest was the development of the aromatic nitrogen mustards, of which N,N-bis(2-chloroethyl)-2-naphthylamine, II, R = 2-naphthyl, was the first (83). It was found to

inhibit several animal tumors and was seen to depress the bone marrow less severely than the aliphatic nitrogen mustards. One of the biggest advantages of the aromatic nitrogen mustards is their reduced local and systemic toxicity which permits oral administration.

Two of the more recent and more successful aromatic nitrogen mustards from the Haddow group are N,N-bis(2-chloroethyl)-p-aminophenylbutyric acid and N,N-bis(2-chloroethyl)-p-aminophenylalanine (16, 5).

From what is already known chemically and biochemically, a tentative interpretation of the reaction mechanism can be made. Methylbis(2-chloroethyl)amine, III, in aqueous solution at temperature, pH,



and dilution corresponding to biological conditions, gives the cyclic immonium cation, IV. This type cation is highly reactive and readily combines with water to yield methyl-(2-chloroethyl)-(2-hydroxyethyl)amine, V. The remaining chloroethyl group can repeat this

process to give methyl-bis(2-hydroxyethyl)amine. The cyclic immonium cation is the biologically reactive compound. It is capable of alkylating such groups as the -SH group of proteins to give a derivative of the type, VI.

Dividing cancer cells go through the mitotic cycle at an increased rate, as compared to the normal cell. Anything which might hinder this process, especially by interference with the mitotic cycle, might be important as an antitumor agent. It has been observed that cells treated with nitrogen mustard exhibit lagging chromosomes. The possibility of a relation between alkylation by nitrogen mustard and this phenomenon has been pointed out by Goldacre et al. (74). He further hypothesized this phenomenon as important for a chemotherapeutic effect.

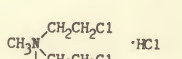
Ross (181) and Everett (59), on the other hand, questioned whether the action of the aromatic compounds could be related to the formation of the immonium cation intermediate. Ross (182) pointed out that since the aromatic amines are weaker bases than the aliphatic amines, and since the normally non-bonding electron pair of the nitrogen atom is less available, the aromatic nitrogen mustards should be less likely to cyclize. Moreover, if they did cyclize, the immonium cation produced should be much less stable than that from the aliphatic nitrogen mustards. Since, in the aromatic nitrogen mustards, no evidence was found for a cyclic immonium cation, Goldacre et al. (74) suggested that a double carbonium ion was formed by the removal of two chloride ions, leaving a doubly positive charged intermediate

which was postulated to be the reactive agent. This was thought to give rise to a cross-linkage with protein or nucleoprotein. The hypothesis of such a linkage led Goldacre to suggest that possibly this cross-linkage could be the cause of the failure of dividing chromosomes to separate upon prior treatment with nitrogen mustard.

This theory was criticized by Biesele et al. (205) and by Loveless and Ross (116) who showed that monofunctional compounds, that is those amines containing a single 2-chlorethyl-group, were also active against tumors and in causing chromosomal aberrations.

It is thus generally agreed that the biological activity of the alkyl nitrogen mustards is due to the reactivity of the cyclic immonium cation with important chemical units within the cell. A growing amount of evidence suggests that the nucleic acids may be the most important biological site of reaction of the immonium cation (70, 182).

A variation of the usual nitrogen mustard structure has been found effective in the compound methyl bis(2-chloroethyl)amine N-oxide hydrochloride, commonly called Nitromin, VII. This compound,



VII

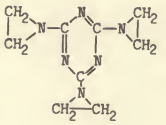
first synthesized by Stahmann and Bergmann (205), was found to be a better antitumor agent than methyl bis(2-chloroethyl)amine in certain animal tumors and also to be less toxic (116). The reaction pattern

of this compound is said to be similar to the aliphatic nitrogen mustards, and methyl bis(2-chloroethyl)amine is even said to be a possible intermediary metabolite by the loss of the oxygen atom. This latter conversion is not thought to have any effect on the overall antitumor activity of the compound. Nitromin has been used more in Japan than elsewhere.

The chemistry and pharmacology of the aliphatic nitrogen mustards have been reviewed by Gilman and Philips (73, 166) and by Ross (182).

As the theories of the cross-linking of nitrogen mustards and of the presence of the active ethylenimmonium rings progressed, investigators began to look for agents with similar structures and actions.

Structurally, the ethylenimines are closely related to the ethylenimmonium ions. Thus, it was natural to choose 2,4,6-tris(ethylenimino)-s-triazine, (TEM), (triethylenemelamine), VIII. Not only is



VIII

it structurally related to the ethylenimmonium ions, but it has been described as a cross-linking agent for wool.

This compound turned out to be one of the most effective tumor

inhibiting compounds of the ethylenimine type. The ethylenimine groups of TEM are similar to the reactive groups of the nitrogen mustards, but TEM is more reactive at an acid pH and more stable in basic media. It, also, may be administered orally. None of the convulsive or cholinergic effects of the aliphatic nitrogen mustards are detected in animal experiments with TEM (168). It has been shown to be active against several experimental tumors (140, 178, 34).

Karnofsky et al. (119) have reported clinical results on a wide variety of neoplastic diseases. Of thirteen patients with Hodgkins disease, one patient obtained an excellent remission while five experienced clinical improvement. One patient with lymphocytic lymphosarcoma had a good response. Five patients with reticulum cell sarcoma responded poorly, and four with chronic lymphatic leukemia had some improvement. Other similar results were found with other neoplastic diseases.

Haddow and Timmis (84) showed that the property of biological alkylation can be evoked by diethylarylamines which contain on the 2-carbon of the ethyl groups, an aromatic sulfonic acid group bound in ester form. One of the first compounds to show this was N,N-bis-(p-tolylsulfonoxyethyl)-p-chloroaniline, IX. Previously, this

$$p\text{-}Cl\text{-}C_6H_4\text{-}N(CH_2CH_2O\text{-}p\text{-}Tosyl)_2$$

IX

property had been associated only with the nitrogen mustards. In

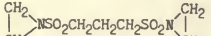
searching for a simplified form of such a molecular arrangement, the compound 1,4-dimethanesulfonoxybutane, X, was discovered. It was found to be a powerful inhibitor of the transplanted Walker carcinoma

$$CH_3SO_2O(CH_2)_4OSO_2CH_3$$

X

in the rat (85). Clinical trials were not too encouraging. These trials, however, did uncover certain facts which led to its use in myeloid leukemia, hence the name, Myleran. The use of Myleran in leukemia has recently been reviewed by Galton (68). Among one group of nineteen cases, remissions obtained ranged from six to twenty-three months.

A structural hybrid of the ethylenimine and the sulfonic ester types has resulted in an agent, 1,3-bis(ethyleniminosulfonyl)propane, XI. Only a limited amount of success has been obtained with this

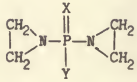


XI

agent (64).

More recently, a series of phosphoramides which involve two or three ethylenimine groupings, have been developed by the American Cyanamid Research Laboratories. These compounds are of the general

type, XII. The letter X may represent a sulfur or oxygen atom, while



XII

Y has been varied with several types of aliphatic, cyclic, or heterocyclic amines.

The first report of the tumor inhibiting properties of N-ethylene-substituted phosphoramides was made by Buckley et al. (27), who demonstrated the effect of triethylenephosphoramide, XII (X = O, Y = ethylenimino), against Sarcoma 180. Since that time, a series of papers (42, 52, 155, 195, 208) have reported the animal screening of compounds of this series.

In these studies it was shown that triethylenethiophosphoramide, XII (X = S, Y = ethylenimino), was considerably more effective than triethylenephosphoramide. Also shown was the fact that substitution of a morpholino-group for one of the ethylenimine groups of triethylenephosphoramide, increased the antineoplastic activity. The compound resulting from such a change is N-(3-oxapentamethylene)-N',N''-diethylenephosphoramide. The clinical results from the use of these compounds have shown a moderate antitumor activity over a wide range of neoplasms. As pointed out by Farber et al. (62), a study of fifteen of sixty-nine patients, with various forms of cancer, showed some

improvement after receiving triethylenephosphoramide. Important improvements were found in patients with Hodgkin's disease, neuroblastoma, and malignant melanoma. The striking disappearance of multiple metastases from a malignant melanoma, as a consequence of the administration of triethylenephosphoramide, marked the first chemotherapeutic effect on that tumor.

Going a step farther, Heidelberger et al. (92, 149, 151, 150, 164), reasoning from the previously mentioned increased activity resulting from the replacement of the oxygen atom of triethylenephosphoramide by sulfur, decided to examine the activity of the compound resulting from the thiation of the active morpholino-N',N''-diethylenephosphoramide, XII (X = O, Y = morpholino). However, no increased activity was found as a result of this change in structure (92). Metabolism studies showed that the thio-compound was dethiated to the previously known triethylenephosphoramide (150).

Currently, the general theory of nitrogen mustard carriers is being developed. That is, the bis(2-chloroethyl)amine-grouping is being introduced into known biologically active systems. These carriers are known to have a tendency to localize in certain organs or tissues more strongly than others. By coupling these with the nitrogen mustard unit, it is hoped to elicit an antitumor action at a selected site. Recent examples are found in those compounds resulting from the replacement of the diethylamino-group of chloroquine and pamaquine by the bis-chloroethylamine side chain. Since one of the active sites of such antimalarials is the liver, these carriers would

then be expected to transport the nitrogen mustard unit to the liver, where, should a tumor be present, the compound could exert its antitumor effect. However, in man, tumors of the liver are less important than many other types of cancer. Also, it is well known that the liver is one of the most active detoxification centers of the body. Thus, the effects of such compounds probably would not be so important as at first anticipated.

2. General Cell Poisons.—The compounds which make up this class of chemotherapeutics are the least specific of all chemical agents used against cancer. Arsenic and benzene are two members of this class which have already been discussed.

The cytotoxic polysaccharides are usually considered under this heading, but will be treated later.

A drug with an action partially resembling that of the cytotoxic polysaccharides is colchicine. In vitro it is active at concentrations as low as one part in 100,000,000 (143).

In the normally dividing cell, the number of chromosomes doubles. The chromosomes move to the opposite ends of the cell, but remain attached by strands called the spindle. This structure upon contraction and rupture permits the formation of two new cells, each having the same number of chromosomes as the parent cell. Colchicine acts by destruction of the spindle, preventing the formation of the two new daughter cells (144). The chromosomal material evidently forms as usual, but no spindle is observable (143). The remaining cell then possesses a double number of chromosomes, called diploidism.

Doubling of chromosomes can be repeated several times leading to polyploidism.

The mitotic inhibition of colchicine naturally suggested its use as a chemotherapeutic agent for cancer. Unfortunately, the drug is not sufficiently specific for tumor use. It destroys dividing cells in the crypts of the small intestine, lymphoid tissue, and bone marrow, and in the mouse is more effective against these tissues than against an implanted Sarcoma 37.

Colchicine can produce a temporary regression in certain kinds of tumors (144, 142). At the high dose levels required, hemorrhage is induced in susceptible tumors, and thus regression is probably not directly a result of mitotic arrest, but rather due to the destruction of the newly formed capillary endothelium in the tumors similar to that found with the bacterial metabolites. Colchicine causes a temporary fall in the number of leucocytes in leukemia, but this is followed by a great rise of the white blood cell count (53).

Demecolcin is a natural alkaloid isolated from Colchicum autumnale (185), differing from colchicine only in the attachment of a methyl group instead of an acetyl radical to the amino group. The advantage of this agent lies in its decreased toxicity, reportedly only one-thirtieth to one-fortieth as high as colchicine (157). Ten patients with chronic myelogenous leukemia, all obtained good remissions from treatment with this compound. Three of six patients with acute and subacute myeloid leukemia showed good responses, but two chronic lymphatic leukemia patients became worse. Twelve other

patients with various forms of carcinoma and sarcoma received no improvement.

Podophyllin is a product from the May Apple or Mandrake root, which produces damage to tumors after a single injection (4). It was first considered for testing because of earlier reports that it caused the production of mitotic figures similar to those from Colchicine (125). Shear and his associates (134, 188), upon separating podophyllin into fractions of different activity towards Sarcoma 37, found it to consist of the four active components, α- and β-peltatins, podophyllotoxin and quercetin. The last compound was less active than the preceding three.

Podophyllin lengthens the survival period of leukemic mice. Generally, the maximum tolerated dose is much higher than the minimum effective dose, but the response depends on the tumor type and host.

For further information of the chemical composition and biological effects of podophyllin, the reader is referred to the recent thorough review by Kelly and Hartwell (121).

3. Hormones.—All cancer is not necessarily autonomous and self-perpetuating. Some tumors remain sufficiently like the tissue from which they developed, to function like the tissue of origin. When a tissue is hormone-dependent, the tumor developing from this tissue will likely be similarly dependent. Both will then atrophy upon removal of hormonal support, and both will likewise flourish with the addition of the necessary hormone. When such normal cells concentrate certain chemicals, then the related tumor may have the same

property (108, 112).

Subsequent discussion will be limited to prostatic and mammary cancer as treated by estrogens and androgens.

Since most prostatic tumors are dependent upon hormonal support, the treatment of this type cancer has developed from a knowledge of the physiology of the prostate gland (111). The prostate gland is activated by the testicular hormones, and consequently orchiectomy abolishes the prostatic secretion and the gland atrophies. The prostatic secretion is eliminated by the administration of estrogens (109) with a resulting reduction in size of the organ. In castrated or estrogenized dogs, the administration of testosterone or related compounds returns the prostate to normal. Prostatic tumors in senile dogs were found to shrink upon orchiectomy or treatment with estrogen, whereas testosterone accelerated their growth.

Since prostatic cancer also generally remains under hormonal control in man, both orchiectomy and estrogen treatment (110, 112) are effective in controlling cancer of the prostate. Conversely, the administration of testosterone accelerated the growth of such tumors.

The first series of patients treated by orchiectomy (112) consisted of twenty patients with wide-spread metastases. Four of these survived for longer than twelve years. Nesbit and Baum (160) reviewed the effects of orchiectomy and estrogenic substances on 1,818 cases of prostatic cancer and stated that patients treated by both methods live longer and more comfortably than non-treated patients.

The progress of treatment of mammary cancer has followed a

similar path. In 1896, Beatson (10) found that removal of the ovaries caused regression of certain mammary tumors. In 1916, Lathrop and Loeb (130) showed that ovariectomized mice developed fewer spontaneous mammary carcinomas in high cancer incidence strains. Lacassagne (129) demonstrated that the administration of estrogenic hormones to male rats of high cancer strains increased the incidence of mammary tumors. This evidence prompted Lacassagne to suggest the use of androgens for therapy of cancer of the breast. Subsequently, it was found that testosterone significantly reduced the incidence of mammary carcinomas in mice of high cancer strains (69, 93). Androgenic treatment of patients with mammary carcinomas has been used with some success (120).

Paradoxically, some benefit has been obtained from the administration of estrogens to patients with mammary cancers. The use of estrogens here was introduced by Haddow et al. (86). In ovariectomized mice bearing mammary tumors, a biphasic effect of estrogen is noted (113). Small amounts of estrogens promote the growth of this tumor, while large amounts suppress its growth.

The age factor is one which is important in hormonal treatment of cancer. Gellhorn (70) pointed out that estrogens should be limited to patients of at least five years postmenopausal. Premenopausal treatment with estrogens may lead to a rapid progression of breast cancer.

In castrated animals, the anterior pituitary attempts to maintain a balanced hormonal system. The gonads being removed, no secretive response can be made. The adrenal glands, however, are affected

by the pituitary secretions. The adrenal glands can produce sufficient quantities of growth promoting steroids to maintain dependent neoplasms. For this reason, adrenalectomy is another method used in combatting mammary cancer. Further, since the pituitary is also involved in this hormone system, its removal has been adopted. This once intricate operation has been skillfully simplified by Lulft et al. (145), and definite improvement has been noted in patients treated by the removal of this endocrine gland.

Overall, tumor control by hormonal means is therefore a matter of hormonal imbalance. The means to attain this end are the removal of the natural source of the tumor supporting hormone, surgically and/or by means of anti-hormones.

4. Antimetabolites.—An antimetabolite is a substance which will stop a metabolic process by removing from the process a necessary intermediate. Antimetabolites generally are structural modifications of the compounds whose metabolism they inhibit. It is thought that an antimetabolite is accepted into a given biochemical process and, because of its difference in structure, is trapped and hence blocks the metabolic process. In the case of the cancer antimetabolite, the result desired is the death or marked inhibition of growth of the malignant cell.

The experimental basis for such a theory lies in the phenomenon of competitive antagonism between metabolite and antimetabolite. The original work on this topic was first published by Woods and Fildes (223, 65). It was shown that the essential compound p-aminobenzoic

acid could be replaced in the diet of streptococcal bacteria by sulfanilamide. The latter compound, however, could not be converted into the essential citrovorum factor, and hence the metabolic pathway was blocked. This concept is now generally accepted and has been treated in several reviews (225, 220, 174).

It should be pointed out that, in the bacterium there is an essential requirement for p-aminobenzoic acid which is absent in the animal cell. This requirement thus renders the bacterium vulnerable to p-aminobenzoic acid deficiencies, which in turn have no harmful effects on the animal cell. Hence, the difference between a bacterium and a mammalian cell is much greater than that between a cancer cell and a normal one. Thus, one might expect more difficulty in finding a useful cancer cell antimetabolite.

While there are no known qualitative differences between normal cells and cancer cells, there are quantitative differences. Neoplasms have considerably lower metabolite, cofactor, and enzyme concentrations than most normal tissues (8, 96, 161, 221). Proceding from such findings, Shapiro and his associates have concentrated on chemotherapeutic methods which take advantage of such quantitative differences. The reasoning used here is that an antagonist will decrease a cellular reaction by the same proportion in a tumor cell and in a normal cell. Since the normal cell has a greater abundance of metabolites, cofactors, and enzymes, the reduction will not be so critical here as in the tumor cell. Thus, Shapiro and coworkers have studied the use of the vitamin B_6 antagonist desoxypridoxine (194), the

riboflavin antagonist 6,7-dimethyl-9-hydroxyl-isoalloxazine (191), and the niacin antagonists 2-ethylamino-1,3,4-thiadiazole and 6-amino-nicotinamide (192). Each of these agents has been found to have moderate antitumor activity against a variety of experimental tumors.

The early use of cytotoxic polysaccharides has been pointed out. This material was originally classified as a general cell poison. As time passes, however, the importance of carbohydrate metabolism in the cancer cell is attaining more recognition.

Warburg (214) has proposed that the carcinogenic process is caused by an irreversible injury to cellular respiration and subsequent excessive fermentation. His correlations of respiratory depletion, as a result of the action of polycyclic hydrocarbons on cellular mitochondria, is indeed thought provoking. If these fermentation processes are as important as Warburg suggests, the cytotoxic action of certain polysaccharides may not be so mystifying as previously thought to be.

a) Amino acid antimetabolites.—The compounds which make up this class are said to act by interfering with the metabolism of an amino acid. In attempting to choose an effective antimetabolite, an analog must be picked which will interfere with an analogous metabolite which carries out an important biochemical reaction. Methionine is an amino acid which takes part in important one carbon unit transfers. Antimetabolites of methionine would thus be expected to have marked physiological effects.

Ethionine is the ethyl analog and hence a supposed methionine

antimetabolite. The structure of this compound is shown below, XIII.

XIII

It had been shown to produce liver and pancreatic damage in animals (49, 90, 75) and to inhibit the Jensen sarcoma in rats (138). Skipper et al. (201) have noted a synergistic effect with this compound and Methotrexate in transplanted mouse leukemia. Since it has been observed that, in the presence of an excess of certain amino acids, an inhibition of tumor growth may result, Levy et al. (139) studied the effect of ethionine on the free amino acid levels in normal and tumor-bearing rats, with inconclusive results. White and Shimkin (222) studied the effects of this compound on six patients with advanced cancer. On a methionine deficient diet, five to ten grams of ethionine daily produced toxic results, such as impaired hepatic function, diarrhea, rash, and occasionally renal damage. No significant antitumor effects were observed.

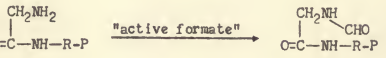
The leukocytes of acute leukemia and chronic myeloid leukemia exhibit a more rapid turnover of radioactive L-cystine than do normal leukocytes (218). A decrease in the availability of L-cysteine or L-cystine would therefore have important effects on leukocytes of leukemic patients. Advantage has been taken of such facts in the use of selenium cystine, XIV, for treatment of leukemia (219). Two acute

XIV

leukemia patients and two with chronic myeloid leukemia were treated orally with this compound. All patients showed a rapid decrease in total leukocyte count as well as a decrease in spleen size. These effects were seen even in a patient who had become refractory to the best agents available today, 6-mercaptopurine and Aminopterin. The most striking and consistent effects were seen in acute leukemia. The nausea and vomiting associated with the oral use of this compound prevented repeated dosage in attempts to determine whether an appreciable clinical remission could be obtained.

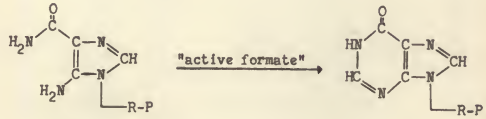
b) Antagonists of de novo synthesis of purines and pyrimidines.—The compounds which make up this class inhibit the formation of the purine or pyrimidine rings by their action on one carbon unit incorporations or by their glutamine antagonism.

Compounds which block the formylation of purines, do so at the expense of two reaction sites. The first is the formation of formylglycinamide ribotide, XVI, from glycinamide ribotide, XV, by formate



XV XVI

incorporation. The second reaction is the formation of inosinic acid, XVIII, from 4-amino-5-imidazolecarboxamide ribotide, XVII, by the same

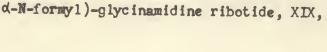


XVII XVIII

mechanism. The formation of inosinic acid has been postulated as involving a 5-formamido-4-imidazolecarboxamide ribotide intermediate (216). Proof of the formation of this compound is not available at present.

The "active formate" of such transformations is supplied by the citrovorum factor or a closely related derivative, anhydroleucovorin (81). The mechanism of the action of those agents which inhibit these conversions has been demonstrated to be an inhibition in the conversion of folic acid to citrovorum factor, or anhydroleucovorin, and its subsequent conversion to a slightly different form called coenzyme Z, which is the active formylating agent (28).

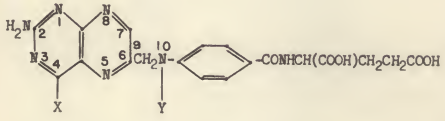
The reaction site at which glutamine antagonists interfere, is that at which (α-N-formyl)-glycinamidine ribotide, XIX, is produced



XIX

from formylglycinamide ribotide, XVI. These compounds do this by reducing the availability of glutamine, and hence the amide nitrogen of glutamine, which is transferred to XIX.

The most important inhibitors of the formylation reaction are the derivatives of folic acid (pteroylglutamic acid, PGA), XX (X = OH, Y = H). A preparation of folic acid was reported to be capable of



XX

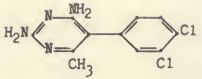
causing complete regression of thirty per cent of spontaneous breast tumors in mice (135, 136). Subsequent chemical work identified the agent as the Lactobacillus casei factor, pteroyltriglutamic acid, or teropterin (141). Farber et al. (60) then tried teropterin and dioopterin on ninety patients, all of whom showed no objective evidence of improvement. Acute leukemia in children was apparently accelerated by these materials. From the tumor promoting effects of these folic acid derivatives, Farber envisioned an antifolic action by analogs of folic acid. In 1948 Farber et al. (61) reported that 4-aminopteroylglutamic acid (Aminopterin), XX (X = NH_2, Y = H), induced temporary remissions of acute leukemia in children. Since then, it has been found that 4-amino-N^{10}-methylpteroylglutamic acid, called Amethopterin

or Methotrexate, XX (X = NH_2, Y = CH_3), in certain children will also temporarily eradicate all signs, symptoms, and laboratory evidence of acute leukemia (190). These two compounds represent the most useful anti-folic compounds available. As was mentioned earlier, the end result of this inhibition is the lack of incorporation of the one carbon fragments into the purine molecule. The inhibited carbon atoms would become carbon-2 and carbon-8 upon their eventual incorporation into the purine ring system.

Although failure of purine ring formation is said to be responsible for the chemotherapeutic effect of the folic acid antagonists, it should be pointed out that there are other biochemical results from their use. Thus, folic acid deficiency induced either by diet or 4-amino antagonists of folic acid, prevent the incorporation of formate into the 5-methyl position of thymine (77) and into the β-carbon of serine (169).

A second general type of active folic acid antagonist is found in those compounds in which the 9-carbon or 10-nitrogen of pteroylglutamic acid have been substituted with a methyl group. In mice with transplanted leukemia, they have produced some increase in survival time (217).

A third type of folic acid antagonist lacks the pteroylglutamic acid skeleton. Structurally, they are diaminodichlorophenyl pyrimidines. The most important member of this group is 2,4-diamino-5-(3',4'-dichlorophenyl)-6-methylpyrimidine, XXI. This compound acts through the same mechanism as the 4-amino-derivatives of pteroylglutamic acid (104). However, the inhibitory effect on L. citrovorum

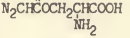


XXI

is reversed only slightly by citrovorum factor. Only in the case of acute toxicity of this compound in animals and man, can a reversal by citrovorum factor be demonstrated (89, 158). In children with acute leukemia, the compound induced complete remissions in two out of twelve previously untreated patients. Seven children previously treated with Methotrexate failed to respond to treatment with this compound. No improvement in adult therapy has been noted (62).

Since the compound accomplishes nothing which Methotrexate cannot do, and since it is much more toxic than Methotrexate at therapeutic dose levels, it has been considered an impractical agent in the treatment of leukemia (158). Similar results have been reported for the 4,6-diamino-1-aryl-1,2-dihydro-s-triazines, which structurally are closely related to the above pyrimidine compound (62).

As pointed out earlier, glutamine antagonists inhibit the introduction of nitrogen-3 of the purine skeleton. A substituted serine, O-diazoacetyl-L-serine (azaserine), XXII, isolated from a



XXII

bacterial filtrate by a group of investigators from Park-Davis (9), was shown to inhibit the above purine conversion (137, 91) and to have a marked antitumor effect against Sarcoma 180 (206) as well as several strains of mouse leukemia (32). As yet, no therapeutic application for azaserine alone has been found. Its use in conjunction with other agents will be discussed later.

c) Antagonists of purine and pyrimidine incorporation into nucleic acids.—In an attempt to find an exploitable biochemical difference between cancer cells and normal cells, many investigators have turned to the study of analogs of the natural-occuring purines and pyrimidines. This has been based on several well known facts. Cancer cells are produced from normal cells, and once a cancer cell has been produced, the daughter cells are also cancerous. Modern genetic theory tells us that inherited traits are carried in the genes and chromosomes. The carriers of genetic information are nucleic acids, which, among other groupings, contain purines and pyrimidines. Abnormal nucleic acid metabolism, and hence purine and pyrimidine metabolism, might, therefore, be expected in cancer cells. However, such generalizations often tend to overlook the problem of specificity. Whatever is qualitatively said concerning nucleic acid metabolism of the cancer cell, can be said of the normal cell as well.

Skipper (199) has pointed out that if one accepts the view that the microscopic appearance of cells, their invasiveness and mitotic rates, are the manifestations of a myriad of orderly intracellular events, then one must assume that such cells differ because of some

qualitative or quantitative biochemical or biophysical differences. This makes it appear reasonable to expect that tumor cells would be unlike their normal precursors in appearance, behavior, and most important of all, response to chemical inhibitors. From this idea of differences between cancer cells and normal cells, investigators have proceeded to build an antimetabolite theory in terms of nucleic acid metabolism.

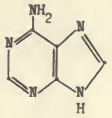
The first step in developing a working theory was to investigate the possibility of a definite heterogeneity of tissue nucleic acids. Rhoads (170), in a review of the literature, has adequately established the heterogeneity of nucleic acids in terms of a purine-nucleic acid theory in cancer chemotherapy.

Adjusting the previously mentioned antimetabolite theory to the purine-nucleic acid picture, one obtains a working hypothesis, which in many respects very closely fits experimental data. Thus, certain preformed purine molecules are known to be incorporated into nucleic acids. The theory would then propose the incorporation of altered purines to give abnormal nucleic acids, which in turn might lead to the destruction of the tumor cell by the synthesis of non-viable chromosomal material. Incorporation of purines into nucleic acids is not, however, the entire story. Some purines are physiologically active, although they are not known to be incorporated into nucleic acids to any appreciable extent.

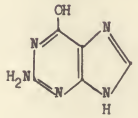
It is interesting to note that, of the purine and pyrimidine bases in nucleic acids, the purines are incorporated to a much greater

extent. This is a possible explanation for the much greater success of purine than pyrimidine antimetabolites in cancer chemotherapy (176). Subsequently, only analogs and derivatives of purine compounds will be considered.

The nucleic acids of living cells are found to contain two purine compounds, 6-aminopurine, called adenine, XXIII, and 2-amino-6-hydroxypurine, called guanine, XXIV.

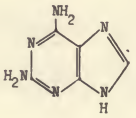


XXIII



XXIV

The first purine antimetabolite to be studied was 2,6-diaminopurine, XXV. It was demonstrated to act as a precursor of nucleic



XXV

acid guanine in the rat (12). It also was shown to have a selective effect against Sarcoma 180 cells grown in tissue culture at levels

which did not harm normal cells (21) and to prolong the survival time of mice with transplanted leukemia Ak 4 (29).

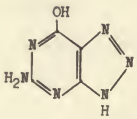
Little is known about the actual metabolism of 2,6-diaminopurine. At one time, this compound was thought to be an intermediate in the conversion of adenine, XXIII, to guanine, XXIV (12). Recent work in bacterial systems, has shown that this pathway is no longer tenable (148). It has been suggested (148), that the metabolism of this compound may involve a transformation to the 8-hydroxydiamino compound analogous to the oxidation of adenine to 2,8-dihydroxyadenine in the rat (11) and in man (207).

This compound has been found to be toxic at dose levels at which other purine derivatives are not toxic. The maximum tolerated dose is approximately ten mg./kg. daily (30). In a report on leukemic patients, one patient received three separate remissions over a period of four years. Two others appeared to be somewhat helped, while twenty-six others similarly treated were not benefited at all (30). Because of the severe nausea, vomiting, and rarity of action against clinical leukemia, it does not seem to be a very practical agent for use alone.

There seems to be a marked species difference in the incorporation of guanine into nucleic acids. The rat incorporates extremely small amounts of this compound (7), while C57 Bl mice utilize guanine to a greater extent (26). In a series of experiments with a spectrum of mouse and rat tumors grown in rats and hamsters, it was found that relative to normal tissues, all tumor tissue utilizes guanine very

poorly for the synthesis of nucleic acid guanine (15). It has been pointed out that this may be due to a high tumor guanase level (15), which would thus form xanthine and the resulting nucleic acids would contain no incorporated guanine. However, no difference has been found between tumor and normal tissue levels of enzymes that degrade guanine and its derivatives (100). Supporting the theory of guanase degradation is the fact that 4-amino-5-imidazolecarboxamide, a supposed guanase inhibitor, causes greater incorporation of guanine into nucleic acids (15).

In view of the specificity and other characteristics of guanine, analogs of guanine have been selected as possible antitumor compounds. One of the most important of such compounds, 8-azaguanine, XXVI, was first synthesized by Roblin et al. (175) and has been widely



XXVI

tested as an antitumor compound. Kidder et al. demonstrated it to cause marked growth inhibition in certain mouse tumors and leukemias (123, 124), as well as in Tetrahymena geleii (122).

Kidder et al. (123) and Armistead et al. (6) described the use of this agent in seven patients, revealing that they all developed

toxic dermatitis and frequent gastrointestinal symptoms, but no clinical improvement.

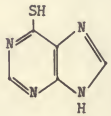
As in the case of guanine, more 8-azaguanine is incorporated into nucleic acids when accompanied by 4-amino-5-imidazolecarboxamide treatment. An inhibition of guanase by this latter compound is again indicated (35). It has been shown that the enzyme guanase, which deaminates guanine, also can deaminate 8-azaguanine (183).

The mode of action of 8-azaguanine may thus be through a reduction of the availability of guanine by inhibiting guanine utilization (124), or through incorporation into nucleic acids to give fraudulent nucleic acids. These latter materials would then be the active pharmacological agents (94, 152). More recently, the inhibition of adenosine deaminase has been proposed as the mode of action of 8-azaguanine (63).

Flavotin, 9-(1'-D-sorbityl)-isoalloxazine, has been found to potentiate the tumor inhibition of 8-azaguanine in C57 mice, although flavotin alone is ineffective (193). Deitrich and Shapiro (44) observed that flavotin antagonized tumor xanthine oxidase in vivo. Liver xanthine oxidase activity, in vivo or in vitro, was not significantly affected. The inhibition of tumor xanthine oxidase by flavotin leads to an accumulation of xanthine. Xanthine, however, inhibits the action of guanase, the enzyme which metabolizes 8-azaguanine to the inactive 8-azaxanthine. Thus, the active 8-azaguanine remains in the body longer and has a greater antitumor effect.

Hitchings and his associates (56, 101, 55, 103), recognizing

the importance of the purine antimetabolite theory, developed a wide range program. From this study, 6-mercaptopurine, XXVII, was



XXVII

synthesized and shown to be a purine antagonist in the wild strain of Lactobacillus casei.

The antitumor effects of this compound were first demonstrated by Clarke et al. (40, 38) in Sarcoma 180 in mice and later by others (197, 71) in various solid tumors and in tissue culture (19). It was also found (40) that the antitumor effects of 6-mercaptopurine could not be prevented by simultaneous administration of adenine, guanine, xanthine, hypoxanthine, 2,6-diaminopurine, 8-azaguanine, citrovorum factor, or folic acid. In some strains of mouse leukemia it causes considerable prolongation of survival time (197, 131, 76).

Using sulfur-labelled 6-mercaptopurine, Elion et al. (54) have demonstrated that 43.5% of the injected radioactivity can be recovered from the urine of mice in four hours. Chromatographic separation has shown that this activity is made up of 32% 6-mercaptopurine and 39% thiouric acid. In forty-eight hours, 60% of the activity of an injected dose can be isolated from the urine. Incorporation into tissue

nucleic acids was highest in the gut, but also detectable in the liver, spleen, kidney, lung, and sternum, and not detectable in brain tissue. Elion et al. (54) believe that the carbon to sulfur bond is still intact in incorporated 6-mercaptopurine. Similar metabolic results have been reported in man (88).

Skipper (198) has demonstrated that mice bearing Sarcoma 180 and Adenocarcinoma 755, incorporate less formate into tumor and intestinal nucleic acids upon treatment with 6-mercaptopurine. However, there is no change in adenine incorporation under such conditions. These facts, along with other data, have been collected in attempts to determine a mechanism of action of 6-mercaptopurine. In Streptococcus faecalis, 6-mercaptopurine-8-C^{14} metabolism was found to produce a number of compounds which, in two dimensional chromatography, moved to the same region of the chromatogram as known nucleotides (118). Thus, it may be that 6-mercaptopurine activity results from its incorporation into a nucleoside or nucleotide. It was found, however, that 6-mercaptopurine riboside is no more active against Adenocarcinoma 755 than 6-mercaptopurine itself. Such data would tend to detract from the above incorporation theory. Biesele (20) has pointed out that, at least part of the action by which 6-mercaptopurine decreases mitotic incidence in tissue culture, is due to the action of 6-mercaptopurine as an antimetabolite of coenzyme A.

A thorough appraisal of the clinical value of 6-mercaptopurine was made at the Conference on 6-Mercaptopurine at the New York Academy of Sciences in April, 1954 (156). In general, it was found that this

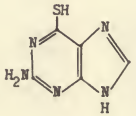
compound will produce remissions in children with acute leukemia. It will also cause remissions in those who have become resistant to Methotrexate. Occasional remissions were reported in adults. Bross (24) in a statistical study of the data presented, indicated that approximately one-third of the children with acute leukemia and one-seventh of the adults will respond to this agent.

The phenomenon of drug resistance is one of the most critical in cancer chemotherapy. There are several agents now available, which, were it not for the eventual resistance to them which the animal body builds up, would furnish effective control of several forms of cancer. It is believed that there are alternate pathways of metabolism in the body which are used as a major pathway becomes blocked.

There are believed to be two sources of nucleic acid purines. The first is the de novo synthesis or synthesis from small molecules. The second is the direct incorporation route, in which preformed purines are incorporated into nucleic acids. Antimetabolites of the purine type are said to inhibit the latter route of nucleic acid synthesis. It has been mentioned previously that azaserine blocks the former pathway of small molecule synthesis of nucleic acid purines. Thus, the use of purine antimetabolites and azaserine together would exert a double block, one to each synthetic pathway. By such treatment, synergistic effects have been obtained. Stock et al. (209) have pointed out that, when two or more anticancer chemicals are used together, there will be less chance for the appearance of mutant forms of tumor cells which are resistant to the combination of carcinostatic agents.

This has been said to be due to the low probability of formation of double or multiple mutants. The differences between cancers in terms of the evolution of drug resistance has been reviewed by Law (132).

A compound closely related to 6-mercaptopurine is 2-amino-6-mercaptopurine or thioguanine, XXVIII. This compound was synthesized



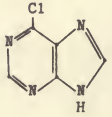
XXVIII

by Hitchings (102) from guanine. Philips et al. (167) reported that this compound was not effective against a 6-mercaptopurine-resistant strain of Sarcoma 180 in mice. It also was reported that it does not produce the characteristic pulmonary edema in the rat or the hepatotoxic or gastrointestinal effects of 6-mercaptopurine in the dog.

Burchenal (33, 31) described its use in three patients with chronic myelocytic leukemia, all of whom responded with remissions. In a report on forty-eight children with acute leukemia, Farber et al. (62) pointed out that thioguanine gave results similar to those with 6-mercaptopurine. The longest remission lasted sixteen months, while the average remission lasted six months. An average treatment period of three to ten weeks was required for the production of remissions. The report of Philips et al. (167) as to the lack of hepatic injury

in the dog, does not seem to hold in man. A dose of two mg./kg. daily produced marked hepatic injury in at least one child. Cessation of the treatment with this compound led to improved liver function.

Another purine compound closely related to 6-mercaptopurine is 6-chloropurine, XXIX. This compound was first synthesized in 1954 by



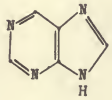
XXIX

Bendich et al. (13). In contrast with 6-mercaptopurine, it has no inhibitory effect in S. faecalis 8043, S. faecalis/A, or L. arabinosus (62). Clark et al. (38) have shown that it does inhibit the growth of Sarcoma 180 in mice. As in the case of thioguanine, it is without effect against a 6-mercaptopurine-resistant strain of Sarcoma 180. Murphy et al. (159) have shown that 6-chloropurine, at twenty mg./kg. daily, will also produce remissions in the acute leukemias and in chronic myelocytic leukemia. Here, also, a cross-resistance to 6-mercaptopurine is found. Thioguanine and 6-chloropurine seem to be indicated only when a severe gastric reaction to 6-mercaptopurine is found.

The compound 6-chloropurine has been quite useful as an intermediate in the preparation of 6-substituted purines, due to the high

reactivity of the chlorine atom.

In searching for active purine antimetabolites, it was only natural that the unsubstituted compound should be tried. Thus, purine, XXX, was shown by Clark et al. (39) to have an inhibitory effect



XXX

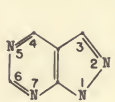
on Sarcoma 180 in mice and to cause an equal inhibition of Sarcoma 180 made resistant to 6-mercaptopurine. Biesele reported that both purine and its riboside were more toxic to cells of mouse Sarcoma 180, than to cells of embryonic mouse skin in tissue culture (22). Hutchison (115) found that forty gamma per milliliter of purine caused half-maximum inhibition of 6-mercaptopurine-resistant and Methotrexate-resistant strains of S. faecalis. Elion et al. (57) also found no cross-resistance to purine in a strain of L. casei made resistant to 6-mercaptopurine.

Few clinical studies have been made on this compound. Most reports involve the use of purine when patients have become resistant to 6-mercaptopurine or Methotrexate. Antineoplastic activity of the compound in man has not been definitely established. The chief disadvantage to the use of this compound has been the severe rash it causes.

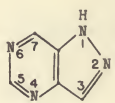
Puromycin is an antibiotic isolated from Streptomyces albonigen The structure for this compound has been determined by Waller et al. (213), who reported the molecule to contain a substituted-6-dimethylaminopurine riboside structure. This compound has been found to inhibit the growth of Trypanosoma equiperdum and Trypanosoma cruzi (95). Troy et al. (211) found that the antibiotic would inhibit the growth of a mouse adenocarcinoma. Haliday et al. (87) reported varying degrees of carcinostatic action produced by fifteen benzilidine analogs of puromycin against mammary adenocarcinomas in mice.

The importance of the purine group has been shown in the demonstration that the carcinostatic activity of puromycin resides in the aminonucleoside portion of the molecule (162).

Within the last five years, Robins and his associates (36, 107, 171, 173) have modified the purine ring in still two other ways. The imidazole portion of the purine ring, XXX, has been synthetically changed to the structures pyrazolo(3,4-d)pyrimidine, XXXI, and pyrazolo(4,3-d)pyrimidine, XXXII. It has been found that various types of



XXXI

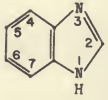


XXXII

amine-substitution of XXXI in position-4 or XXXII in position-7, give

rise to a series of compounds highly active against Adenocarcinoma 755 and Leukemia 5178 (200). Some of these compounds are now being considered for clinical trial.

Benzimidazole, XXXIII, has been shown by Woolley to act as an



XXXIII

antipurine agent in E. coli (224). Gillespie et al. (72) have proposed a guanine antagonism in the physiologic activities of 4-methoxy-6-nitrobenzimidazole. Especially interesting is the reversal of the growth promoting effects of adenine by 4-nitro- and 4-aminobenzimidazole (224, 175). Benzimidazole analogs of the pteroylglutamic acids have been shown to have anti-folic acid activity (50). Other workers have ascribed varying degrees of anti-purine activity to derivatives of the benzimidazole type (99).

Recently, Downing and Schweigert (46, 45) in studies on the role of vitamin B_{12} in nucleic acid metabolism, have found that this vitamin is directly involved in the synthesis of deoxyribose. It is of interest that vitamin B_{12} contains 5,6-dimethylbenzimidazole as part of its chemical structure. Thus, compounds which inhibit the action or metabolism of this unit might be expected to affect the formation of deoxyribose and hence deoxyribose nucleic acids. The

importance of nucleic acid metabolism in chemotherapy has been pointed out previously.

A vitamin which contains a structure similar to that of 5,6-dimethylbenzimidazole is riboflavin. Structurally, riboflavin is 6,7-dimethyl-9(1'-D-ribityl)-isoalloxazine. Compounds which interfere with riboflavin would also be significant in cancer chemotherapy, since riboflavin levels in the livers of animals treated with liver carcinogens are known to be markedly depressed.

A compound closely related to riboflavin is 6,7-dichloro-9-(1'-D-sorbityl)-isoalloxazine. The isoalloxazine ring system of riboflavin remains intact in such a compound, but the methyl-groups are replaced by chloro-groups and ribose by sorbose. This compound was prepared by Holly et al. (106) and found to produce regression of lymphosarcoma in mice.

The relation between these two vitamins, in terms of common antagonists, has been summarized by Gillespie et al. (72) who propose that the benzimidazoles act by inhibiting vitamin B_{12} or riboflavin, or by blocking purine metabolism.

III. STATEMENT OF THE PROBLEM

The importance of purine-type compounds has been shown in the previous section. The carcinostatic activity resulting from the substitution of a nitrogen atom for the 8-carbon of guanine made it seem of interest, therefore, to introduce a more radical change in the structure of such compounds by substituting in this position an element less closely related to carbon and nitrogen. The element chosen was selenium.

The physiological activity of selenium has been known for many years. This was only of academic interest until it was realized that selenium is present in high concentration in plants grown on soils rich in this element and that animals feeding on such plants become affected. This was first reported in 1865 by an army surgeon in South Dakota, although the identity of the active agent was unknown. The relation between such physiological activity and the presence of selenium in plant products was first pointed out by Franke (66, 67).

Selenium, fed as a salt in selenite or selenate form, or in organic combination as in grains, is readily absorbed from the gastrointestinal tract. Solutions of inorganic salts also gain access to the blood stream from parenteral administration. Selenium fumes and dust, as well as hydrogen selenide, are readily absorbed through the respiratory membranes (79).

After absorption, selenium is picked up in the blood and is

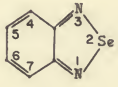
found to be located mainly in the erythrocytes, probably in organic combination with protein (48). It is removed from the blood and deposited in the liver, kidney, and spleen (179). Fifty to eighty per cent of excreted selenium appears in the urine, and eighteen per cent appears in the feces (203).

The exact mechanism of selenium action is not known: however, selenium is found just below sulfur in the periodic table and hence would be expected to be involved in those biochemical reactions in which sulfur plays an important role. Selenate inhibits the growth of yeast, which is reversible by methionine (64). It thus seems quite possible that selenium may take the place of sulfur in a methionine type compound. Indeed, it has recently been shown that selenomethionine is formed by E. coli when selenite is fed into this organism (212). It is also interesting to note that a methionine requiring mutant of E. coli has been shown to use selenomethionine in place of methionine. The incorporation of selenium into cystine to give the carcinostatic agent selenium cystine, has already been discussed in section II B 4 a. Beside the use of selenium cystine, the only pharmaceutically useful form of a selenium compound is found in selenium sulfide. This agent is used topically as a dilute solution in the treatment of seborrheic dermatitis.

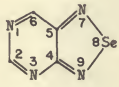
The first report of the natural occurrence of selenium in the animal body, has recently been made by Schwarz and Foly (189). Selenium was found to be a necessary part of a factor which prevents liver necrosis in the rat.

The chemistry of selenium compounds has been reviewed by Painter (163). From this review, it is evident that the investigation of selenium containing compounds has been only meager. Of particular interest is the lack of work on heterocyclic selenium compounds.

The approach used here has been the incorporation of the element selenium into heterocyclic systems which are related to known purine antimetabolites. These compounds have all been derivatives of the ring systems 2,1,3-benzoselenadiazole, XXXIV, or 8-selenopurine, XXXV.



XXXIV



XXXV

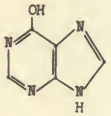
In addition to the change in the 8-position, by the introduction of the selenium atom, another difference from the structure of purine is seen. Both molecules are represented as existing in an ortho-quinone formation. In the case of 2,1,3-benzoselenadiazole, XXXIV, this bond arrangement has been determined (147, 146). Since the 8-selenopurine ring, XXXV, is formed in the same type reaction, it also has been written in this form. Considering the marked physiologic effects found in benzimidazole compounds, this investigation has been carried out with the hope of using the selective physiologic manifestations of the benzimidazole nucleus, accompanied by the added

activity of selenium. It is thus hoped that the derivatives of 2,1,3-benzoselenadiazole will have a specific activity for the cancer cell.

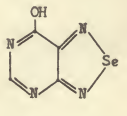
In the case of the 8-selenopurines, the relationship to the purine ring system is much more evident. Depending on the substituent on this ring system, there should be little reason why an antitumor activity could not be expected. Those derivatives which represent natural-occuring purine compounds, with a selenium atom replacing the 8-carbon atom of purine, would be expected to be especially active.

The use of selenium in purine type molecules has received only slight mention in the chemical literature. Recently, Mautner (154) has reported several selenopurines. These, however, had the selenium atom placed outside the purine ring system, and no report was made on the physiological activity of any of these compounds. There is only one reference to compounds containing a heterocyclic selenium atom in a purine nucleus. The compound reported was 1,3-dimethyl-2,6-dioxy-8-selenopurine (184).

The relationship between 6-hydroxypurine, (hypoxanthine), XXXVI, and 6-hydroxy-8-selenopurine, (8-selenohypoxanthine), XXXVII,



XXXVI



XXXVII

is shown above. Hypoxanthine is metabolized by oxidation to xanthine, 2,6-dihydroxypurine. The enzyme xanthine oxidase takes part in this conversion. It would not seem too unreasonable to propose that the same enzyme might oxidize 8-selenohypoxanthine to 8-selenoxanthine. Going one step farther, xanthine is then oxidized to uric acid, 2,6,8-trioxypurine. Recently, Bergmann and Dikstein (18) have proposed that xanthine oxidation to uric acid involves an initial addition of water to the double bond between carbon-8 and nitrogen-9 of the purine ring. Assuming that 8-selenohypoxanthine would be accepted by the enzyme system and hence oxidized to 8-selenoxanthine, it would be quite likely that the subsequent oxidative pathway would be blocked. This can be predicted, since there would be no hydrolyzable double bond available in the selenium compound. The possibility of such a sequence of events is strengthened by the anomalous lack of substrate specificity of xanthine oxidase.

Should such a hypothesis be true, hypoxanthine and xanthine metabolism would be blocked. Since xanthine is known to function as an indirect guanine source (198), the nucleic acid picture might be adversely affected.

The natural purines are bound in nucleic acid formation by an N-glycoside linkage between nitrogen-9 of the purine ring and carbon-1 of ribose or deoxyribose. Considering the 8-selenopurines to exist in a quinoidal form, the 9-nitrogen atom has no available position for bonding into nucleic acids. Koch (126), working with a series of methylated purines in E. coli, found that, while such purines

substituted in the 9-position are not incorporated into nucleic acids, they are active mutagens and are found to inhibit the intermediary metabolism of the cellular nucleic acids.

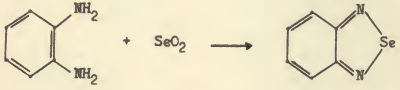
In reviewing the literature, I have noted a previously unmentioned correlation between chemical structure and antitumor activity. If one examines the structure of the active anti-folic acid compounds, he finds that the 4-amino derivatives are the most active. This relationship is widely accepted. However, the 4-position of the pteridine ring corresponds to the 6-position of the purine ring. Several 2-substituted-6-amino purines are known to be very toxic and also to be somewhat selective in their action against tumor cells (22). It has further been noted that the series of active amino derivatives of pyrazolopyrimidines prepared by Robins et al., all have the amino-group located on that position which corresponds to the 6-position of purine.

Considering these facts, the preparation of 6-amino and 6-substituted-amino-8-selenopurines would be highly desirable. The preparation, therefore, of such compounds was undertaken as discussed in the next section.

IV. PROCEDURE

A. DISCUSSION

1. Synthetic Methods.—The method used in this investigation for the preparation of selenium heterocycles is an extension of that reaction used for the preparation of 2,1,3-benzoselenadiazole. This compound was first prepared by Hinsberg (97, 98) by the reaction of o-phenylenediamine with selenium dioxide. Hinsberg named this

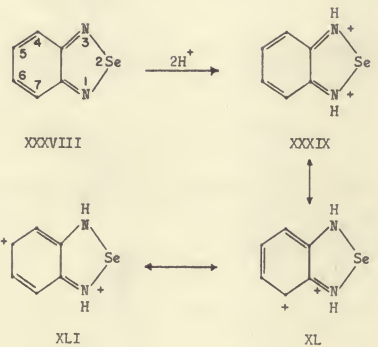


compound piaselenole. In my investigation, selenous acid, rather than selenium dioxide, has been used as a selenium reagent.

a) Derivatives of 2,1,3-benzoselenadiazole.—2,1,3-Benzoselenadiazole, XXXVIII, contains two possible different positions for electrophilic substitution, since positions four and five are equivalent to seven and six. From a consideration of the resonance forms of the di-cationic salt obtained in concentrated sulfuric acid, it can be predicted that nitration should take place at position-4.

The resonance form indicated by XL involves the placement of positive charges relatively close together. Such forms would be expected to contribute less to the reactive form of the molecule than

XLI. This latter form would thus show a positive charge on carbon-5



XXXVIII XXXIX

XLI XL

or carbon-6. Consequently, a higher electron density would be expected at positions-4 and -7, and hence nitration would be directed to these positions. This has been found to be the case. Nitration in concentrated sulfuric acid gave almost quantitative yields of 4-nitro-2,1,3-benzoselenadiazole. The nitration of the similar 2,1,3-benzothiadiazole (51) and benzofurazan (47) also have been reported to take place in this position. As will be shown in the next section, the ultra-violet absorption spectrum of this nitration product and that from the unequivocal 5-nitro-2,1,3-benzoselenadiazole are different. Further proof of the non-identity of the nitration product and 5-nitro-2,1,3-benzoselenadiazole was found in the melting points of

the compounds. The nitration product melted at 219-221° and the 5-nitro-compound melted at 223-224°. The mixture melting point was 183-188°.

In the nitration of the 5-chloro-, 5-methyl-, 5-methoxy-, 5-ethoxy-, and 4,6-dimethyl-2,1,3-benzoselenadiazole compounds, the nitration products have been assigned as 4-nitro-derivatives. This has been based on the quantitative reaction at that position in the unsubstituted compound, as well as the ortho-para directing influence of each of the substituents in the starting materials. Nitration in the seven-position is not probable; at least in the 5-chloro- and 5-methyl-compounds, the products obtained were certainly not the 7-nitro-compound, since the 5-chloro- and 5-methyl-groups were found to be activated in subsequent reactions. Such activation would be unlikely from the 7-position which is meta to the substituents mentioned. In 5,6-dichloro-2,1,3-benzoselenadiazole, the only position available for nitration is the 4-position.

The activation of the 5-chloro-group by the presence of the 4-nitro-group, was used in the preparation of a series of 4-nitro-5-substituted-amino-2,1,3-benzoselenadiazoles by reaction of the chloro-compound with an excess of amines.

Although the analytical results for 5-chloro-4-nitro-2,1,3-benzoselenadiazole were not satisfactory, the good agreement of the analytical data for derivatives of this compound with their theoretical values removes any doubt of the identity of this compound.

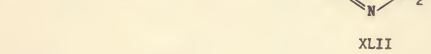
The methyl-group of 4-nitro-5-methyl-2,1,3-benzoselenadiazole

was likewise activated. Use of this compound in a Knoevenagel-type reaction with aromatic aldehydes gave rise to a series of substituted stilbenes.

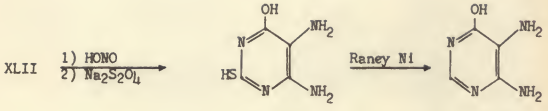
The compound 5-amino-2,1,3-benzoselenadiazole had previously been reported (98). Attempts to prepare 4-amino-2,1,3-benzoselenadiazole, by reduction of the 4-nitro-compound, failed. It was then found that reduction of 4-nitro-2,1,3-benzoselenadiazole by zinc and hydrochloric acid gave 1,2,3-triaminobenzene by reduction of the nitro-group as well as a reductive-cleavage of the selenadiazole ring. Reaction of this latter compound with selenous acid gave the desired 4-amino-2,1,3-benzoselenadiazole. Both the 4- and 5-amino-2,1,3-benzoselenadiazoles were then used to prepare a series of N-acyl-derivatives by standard acid anhydride procedure.

b) Derivatives of 8-selenopurine.—The reaction of selenous acid with aromatic ortho-diamines has been found applicable to the pyrimidine-ortho-diamines.

The compound 4,5-diamino-6-hydroxy-pyrimidine, necessary for the preparation of 6-hydroxy-8-selenopurine, was synthesized by the following series of known reactions (3, 55, 210). Thiourea was

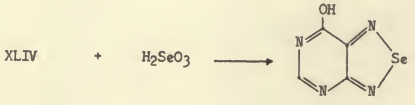


XLII



XLIII XLIV

condensed with ethylcyanoacetate in an ethanolic sodium ethoxide solution to give 4-amino-6-hydroxy-2-mercaptopyrimidine, XLII. This latter compound was nitrosated and reduced to 4,5-diamino-6-hydroxy-2-mercaptopyrimidine, XLIII, which was subsequently dethiated using Raney nickel (25) to give an aqueous solution of 4,5-diamino-6-hydroxypyrimidine (XLIV). This solution was reacted directly with selenous acid to give 6-hydroxy-8-selenopurine, XXXVII.

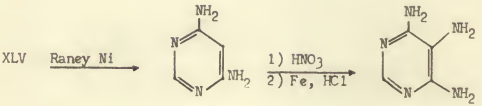


XXXVII

The preparation of 6-amino-8-selenopurine was carried out by reaction of a pyrimidine-ortho-diamine with selenous acid. In this case, the necessary pyrimidine compound was 4,5,6-triaminopyrimidine. Ring closure is theoretically possible in two different positions. However, both ring closures yield the same compound. The necessary pyrimidine-triamine for this reaction was prepared by the following

series of known reactions. 4,6-Diamino-2-mercaptopyrimidine, XLV, was

XLV



XLVI XLVII

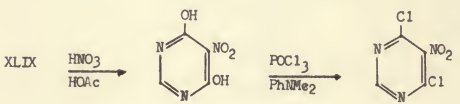
prepared through the condensation of thiourea and malononitrile in the presence of sodium ethoxide (105). This compound was then dethiated to 4,6-diaminopyrimidine, XLVI, (25), which was subsequently nitrated to give 4,6-diamino-5-nitropyrimidine, (25). Upon reduction of this latter compound by the method of Evans et al. (58) a solution of 4,5,6-triaminopyrimidine, XLVII, was obtained which was reacted with selenous acid to give 6-amino-8-selenopurine, XLVIII.

XLVIII

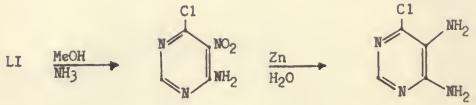
There are three methods whereby substituted-amino groups may be introduced into the 6-position of purine molecules. Reaction of amines with 6-methylmercaptopurines can give the 6-amino-derivatives. This latter compound is prepared by the methylation of 6-mercaptopurine. Attempts were made to convert 6-hydroxy-8-selenopurine to 6-mercapto-8-selenopurine by reaction with phosphorus pentasulfide in tetralin. The selenadiazole ring system is evidently broken in such a reaction, since selenium metal can be seen in the reaction products.

In the absence of the 6-methylmercapto-8-selenopurine, it was decided to attempt to synthesize the 6-chloro-8-selenopurine, which might then be reacted with amines to give amine-substituted-8-selenopurines. As before, the necessary intermediate for the conversion to an 8-selenopurine compound is the ortho-diamine, in this case 6-chloro-4,5-diaminopyrimidine. This compound was synthesized by the following series of reactions. Malonamide was condensed with ethyl

XLIX



L LI

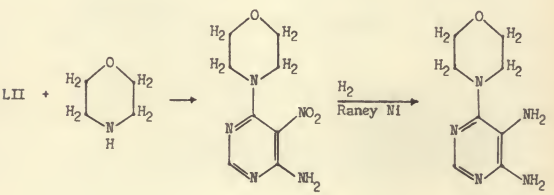


LII LIII

formate to give 4,6-pyrimidinediol, XLIX, by a modification of the method of Hull (114). Nitration of this compound by the method of Boon et al. (23) gave the 5-nitro-4,6-pyrimidinediol, L. Subsequent chlorination using phosphorus oxychloride and dimethyl aniline gave 4,6-dichloro-5-nitropyrimidine, LI. Replacement of the 4-chloro-group by an amino-group was carried out by treatment with methanolic ammonia solution to give 4-amino-6-chloro-5-nitropyrimidine, LII. Reduction of this compound by zinc and boiling water gave 6-chloro-4,5-diaminopyrimidine, LIII (172). Attempts to ring close this compound by reaction with selenous acid gave no identificable product. It is interesting to note that similar difficulties were encountered in the preparation of 6-chloropurine (13). The problem lies in the high reactivity of the 6-chloro-group. In the attempted ring closures of the purine series, hydrolysis to the corresponding hydroxy-compound was consistently found. It is quite probable that similar difficulties were operative here in the attempted preparation of 6-chloro-8-selenopurine.

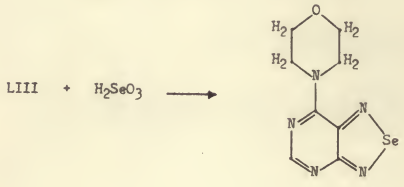
The third method for the introduction of amines into the 6-position of purine molecules is found in the reaction of amines with the previously described 4-amino-6-chloro-5-nitropyrimidine. This

reaction, as well as the subsequent catalytic reduction of the 6-substituted-amino-4-amino-5-nitropyrimidines to the corresponding pyrimidine-triamines, has been described by Daly and Christensen (43). Thus, 4,5-diamino-6-morpholylpyrimidine, LIII, was prepared as shown.



LIII

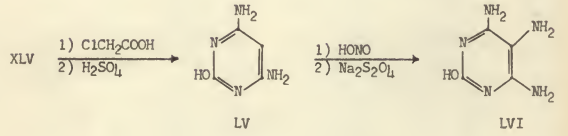
This compound was found to react readily with selenous acid to give 6-morpholyl-8-selenopurine, LIV.



LIV

The compound 2-hydroxy-4,5,6-triaminopyrimidine was prepared

by the following series of reactions. Thus, 4,6-diamino-2-hydroxy-



LV LVI

pyrimidine, LV, was produced from XLV by the action of chloroacetic acid and subsequent hydrolysis of the intermediate 2-carboxymethyl-mercapto-4,6-diaminopyrimidine by sulfuric acid (146). Nitrosation and reduction of LV produced 2-hydroxy-4,5,6-triaminopyrimidine, LVI. This compound was isolated as the amine sulfate (14). The sulfate was found to be incapable of reaction with selenous acid. Therefore, the amine sulfate was converted to the hydrochloride by the action of barium chloride. The resulting solution on reaction with selenous acid yielded an amorphous yellow product. Analytical data showed that the expected 8-selenopurine was not obtained. This product is very insoluble in all organic solvents and water. Mineral acids or bases tend to decompose the compound as shown by the deposition of red selenium upon such treatment. A similar insolubility in water is found in isoguanine (2) which is soluble only to the extent of one part in 16,000 of water at twenty degrees.

2. Ultraviolet-Visible Absorption Spectra.—The absorption spectra of heterocyclic selenium compounds are closely similar to those of the corresponding sulfur analogs except for a shift of

absorption maxima to longer wave lengths (187, 186). This general phenomena has been found to apply here as well. Figures 1, 2, and 3 show a comparison of the absorption spectra of 2,1,3-benzoselenadiazole and 2,1,3-benzothiadiazole in ethanol, 50% sulfuric acid, and 95% sulfuric acid, respectively. Figure 4 shows a comparison of the spectra of 4-nitro-2,1,3-benzoselenadiazole and 4-nitro-2,1,3-benzothiadiazole in ethanol. The same relationship is found here. The values of the wave lengths and intensity of absorption at maximum absorption are listed in Tables 1 and 2.

The 4- and 5-nitro-2,1,3-benzoselenadiazole compounds are found to have spectra which are quite similar. This similarity is shown in Figure 5 and in Table 1. It is thus seen that, at least in ethanol, 4- and 5-nitro-2,1,3-benzoselenadiazole cannot be spectrally separated.

The amino-2,1,3-benzoselenadiazole compounds were found to be particularly well suited to spectral examination. As in the case of aniline and other aromatic amines (202), salt formation of the amino-group with mineral acids gives rise to a spectrum similar to that of the non-substituted compound. In compounds with more than one basic group, this makes possible the study of which groups in a molecule accept an initial proton. The acidification of 4-amino-2,1,3-benzoselenadiazole in 1.2 N hydrochloric acid gives rise to a mono-cation in which the 4-amino-group has been protonated. This is shown by Figure 6. Here it is seen that the spectrum of 4-amino-2,1,3-benzoselenadiazole in 1.2 N hydrochloric acid is very similar to that of

the unsubstituted 2,1,3-benzoselenadiazole in 95% ethanol. In 50% sulfuric acid a dicationic salt is formed in which an additional proton is added to one of the ring nitrogen atoms. The resulting spectrum is thus similar to that of the mono-cation of 2,1,3-benzoselenadiazole. This is clearly shown in Figure 7 and Table 1.

In 5-amino-2,1,3-benzoselenadiazole, a different behavior is found. In dilute acid the first proton goes to a ring nitrogen. This must be true since, had it gone to the amino-group, the spectrum would be like that of 2,1,3-benzoselenadiazole in ethanol. An examination of the data in Table 1 shows that spectrally the electronic form of this compound is quite different from that of the unsubstituted compound in ethanol. In 50% sulfuric acid, however, the second proton is seen to add to the amino group, thus showing a spectrum similar to the mono-cation of the unsubstituted compound. This relationship is shown in Figure 8 and Table 1.

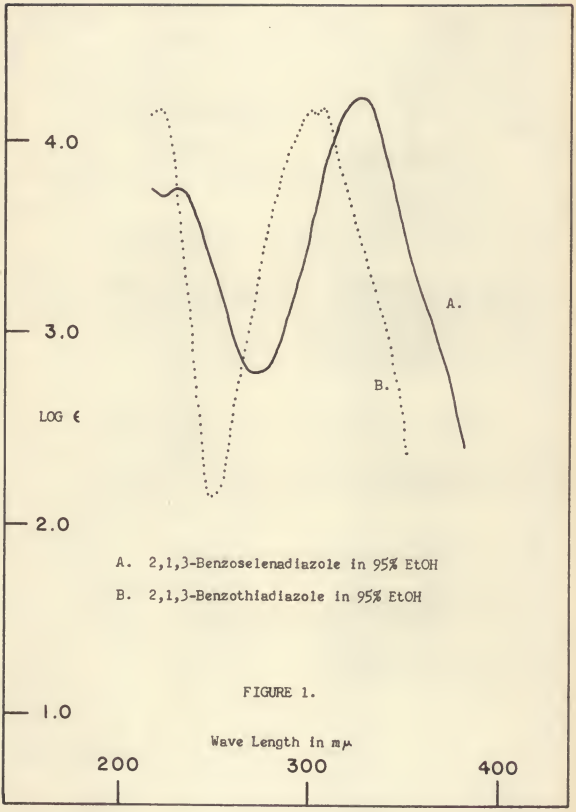
The same phenomenon is observed with 5-N,N-dimethylamino-2,1,3-benzoselenadiazole as shown in Figure 9 and Table 1.

With the small number of 8-selenopurine compounds available, no definite correlations to purine spectra can be made. There are, however, certain similarities in the spectra of purines and 8-selenopurines. Mason (153) has reported the spectra of purines to be made up of two bands. The short wave length band is said to be found only where substituents, such as mercapto-groups, shift the entire spectrum to longer wave lengths. In most other purine compounds, the short wave length maximum lies below the reach of present-day instruments.

Mason has pointed out that one of the important electronic transitions in purines creates a dipole in the direction of the pyrimidine ring, due to the aromatic electron withdrawal effect of the pyrimidine ring. Purine compounds with electron donating groups in the 8-position thus give rise to lower energy activated states and consequently absorb at longer wave lengths.

In the 8-selenopurines, the tendency of the metal selenium to give up its electrons should be greater than that of the 8-carbon of the purine ring system. Hence, in the 8-selenopurines, one would expect transitions, such as that of the purine series, to be of lower energy. It has been found that the absorption maxima of the 8-selenopurines are shifted to wave lengths of about 80 mμ longer than those of the corresponding purines. The data for the absorption spectra of the 8-selenopurines are contained in Table 3.

The absorption spectra were determined with a Beckman Model DU Quartz Spectrophotometer. Sulfuric acid solutions were made up using concentrated sulfuric acid and 95% ethanol. Thus, 50% sulfuric acid refers to a solution of fifty ml. of sulfuric acid and fifty ml. of 95% ethanol. Solutions referred to as 1.2 N hydrochloric acid were 1.2 N hydrochloric acid in 50% ethanol.



A. 2,1,3-Benzoselenadiazole in 95% EtOH

B. 2,1,3-Benzothiadiazole in 95% EtOH

FIGURE 1.

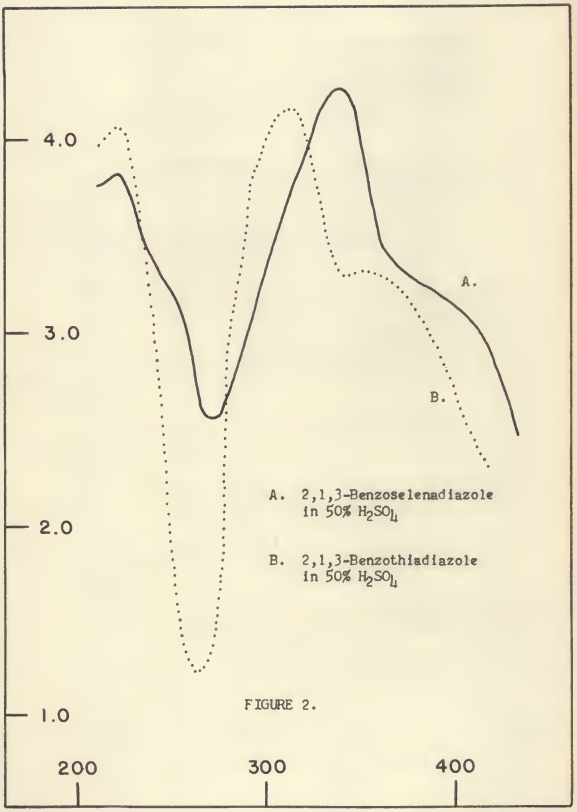
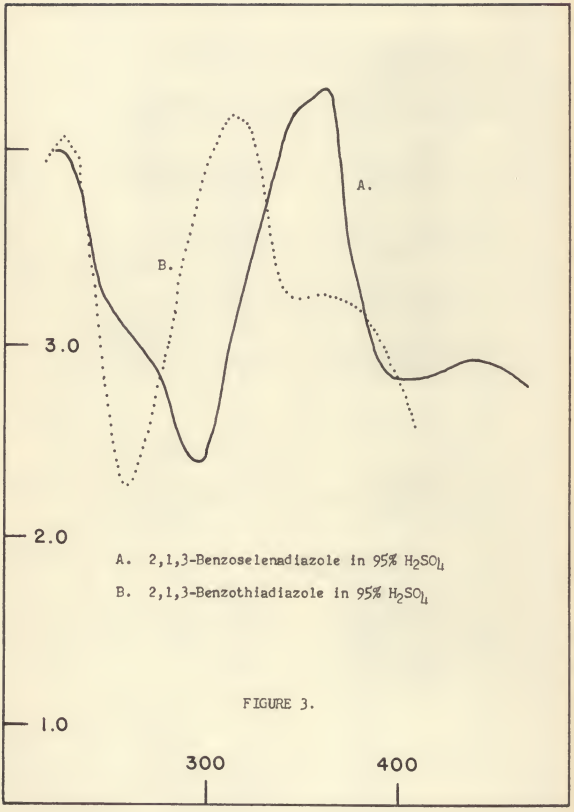

4.0
3.0
2.0
1.0
200
300
400
A.
B.
A. 2,1,3-Benzoselenadiazole in 50% H_2SO_4
B. 2,1,3-Benzothiadiazole in 50% H_2SO_4

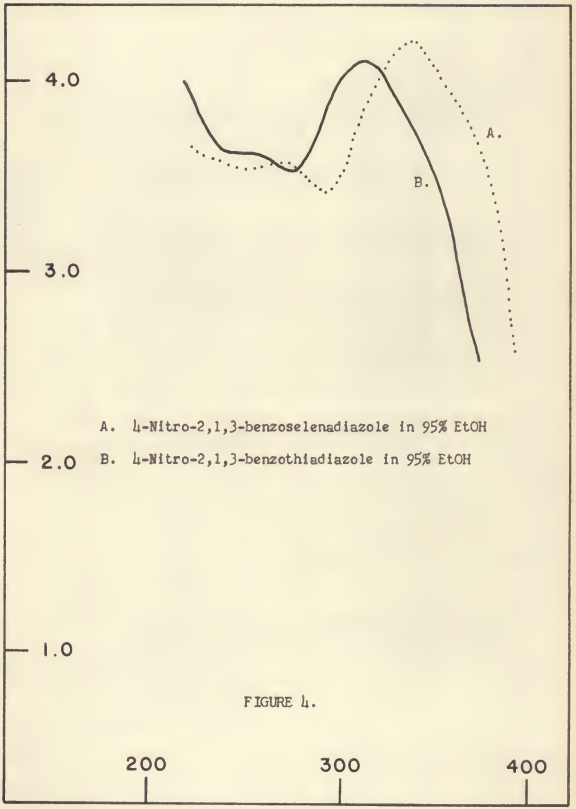
FIGURE 2.



A. 2,1,3-Benzoselenadiazole in 95% H_2SO_4

B. 2,1,3-Benzothiadiazole in 95% H_2SO_4

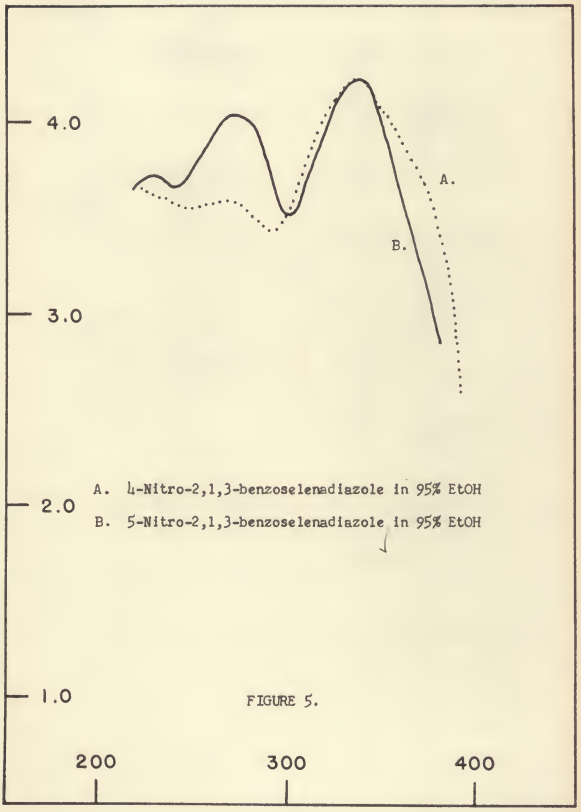
FIGURE 3.



A. 4-Nitro-2,1,3-benzoselenadiazole in 95% EtOH

B. 4-Nitro-2,1,3-benzothiadiazole in 95% EtOH

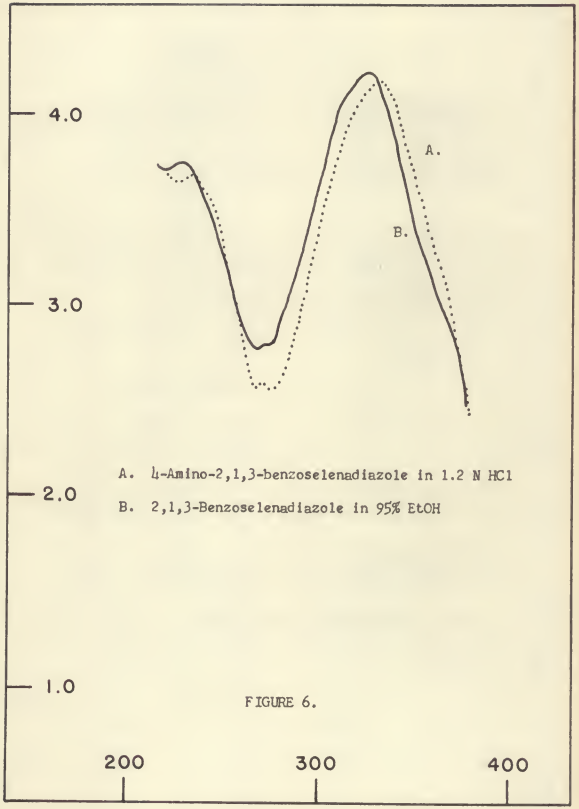
FIGURE 4.



A. 4-Nitro-2,1,3-benzoselenadiazole in 95% EtOH

B. 5-Nitro-2,1,3-benzoselenadiazole in 95% EtOH

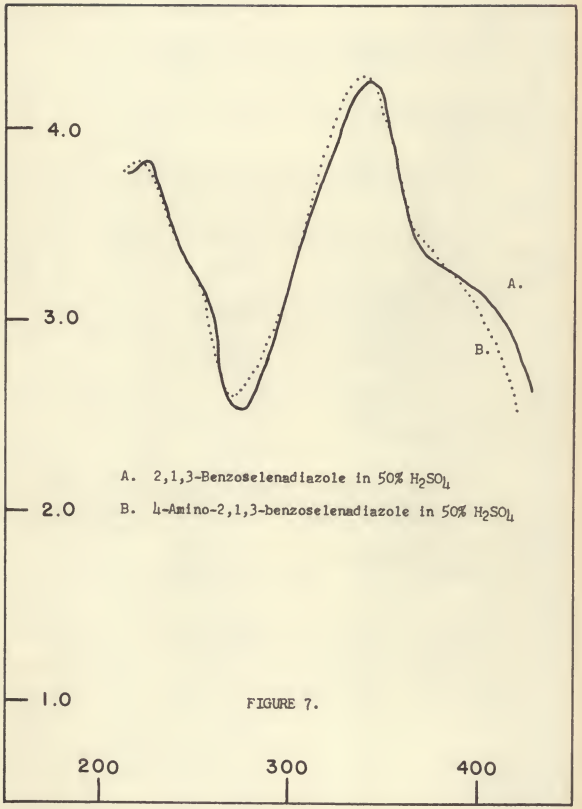
FIGURE 5.



A. 4-Amino-2,1,3-benzoselenadiazole in 1.2 N HCl

B. 2,1,3-Benzoselenadiazole in 95% EtOH

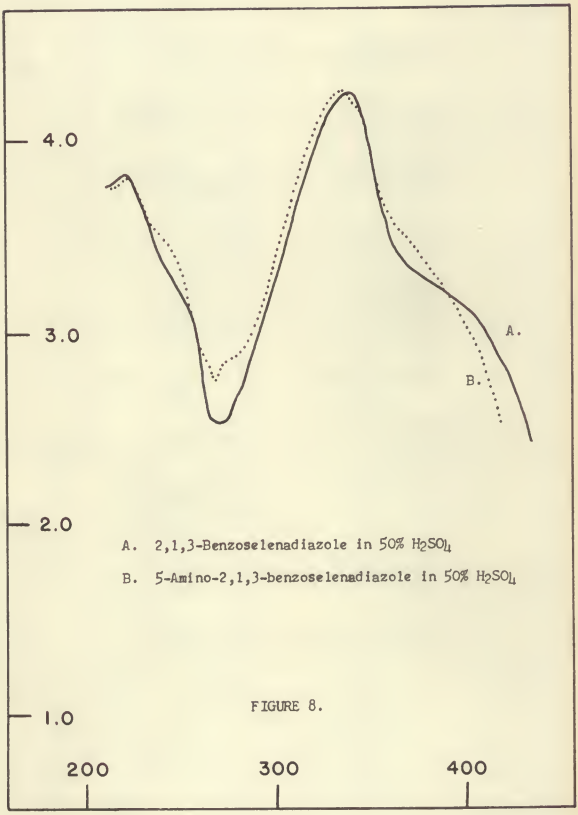
FIGURE 6.



A. 2,1,3-Benzoselenadiazole in 50% H_2SO_4

B. 4-Amino-2,1,3-benzoselenadiazole in 50% H_2SO_4

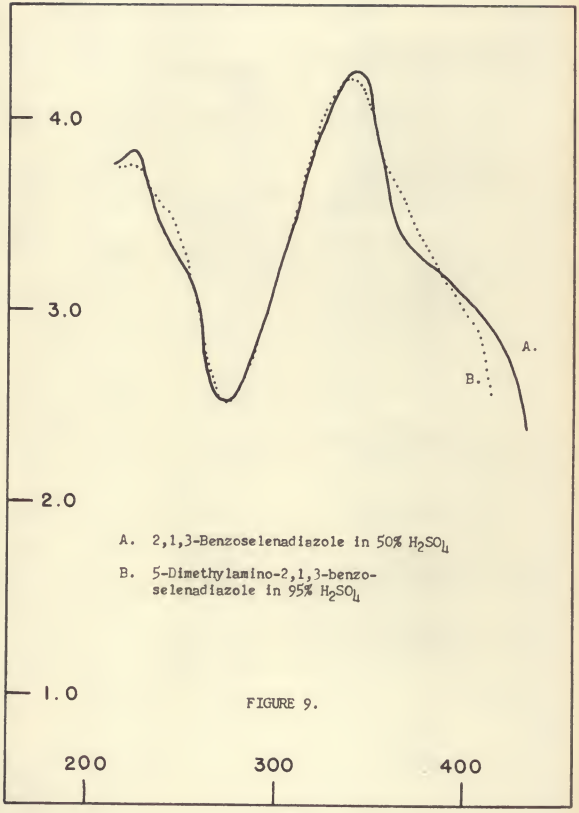
FIGURE 7.



A. 2,1,3-Benzoselenadiazole in 50% H_2SO_4

B. 5-Amino-2,1,3-benzoselenadiazole in 50% H_2SO_4

FIGURE 8.



A. 2,1,3-Benzoselenadiazole in 50% H_2SO_4

B. 5-Dimethylamino-2,1,3-benzoselenadiazole in 95% H_2SO_4

FIGURE 9.

TABLE 1

ULTRAVIOLET-VISIBLE ABSORPTION SPECTRA OF
2,1,3-BENZOSELENADIAZOLE AND
ITS DERIVATIVES

Compound	Values given as λmax in mμ (Log ε)			
	95% EtOH	1.2 N HCl	50% H_2SO_4	95% H_2SO_4
2,1,3-Benzoselenadiazole	232(3.75) 332(4.22)		222(3.66), 250(3.25)[1] 341(4.26) 380(3.29)	220(4.03) 272(2.90) 352(4.29) 362(4.37) 435(2.93)
5-Methyl-2,1,3-benzoselenadiazole	230(3.68) 333(4.24) 365(3.22)		226(3.94) 360(3.08) 347(4.32) 400(3.26)	228(4.14) 282(3.02) 356(4.34) 370(4.42) 438-48(3.09)
5-Chloro-2,1,3-benzoselenadiazole	238(3.62)			
5-Nitro-2,1,3-benzoselenadiazole	230(3.71) 274(4.02) 342(4.21)			
5-Amino-2,1,3-benzoselenadiazole	236(4.11) 328(3.98) 392-6(3.63) 444(3.51)	236(3.89) 333-4(4.09) 390(3.41) 459(3.46)	222(3.78) 338(4.24) 380(3.36)	222(3.82) 337(4.25) 380(3.35)
5-N,N-Dimethyl-amino-2,1,3-benzoselenadiazole	242(4.26) 246(4.25) 280(3.62) 326(3.88) 449(3.83)	224(4.10) 246(3.99) 310(3.79) 334(3.89) 501(3.79)		218(3.75) 242(3.50) 337(4.21) 380(3.31)
4-Nitro-2,1,3-benzoselenadiazole	271(3.57) 339(4.19)			

[1]Underlined wave lengths denote shoulders.

TABLE 1—Continued

Compound	Values given as λmax in mμ (Log ε) 95% EtOH	1.2 N HCl	50% H_2SO_4	95% H_2SO_4
4-Amino-2,1,3-benzoselenadiazole	242(4.14) 330(4.04) 462(3.28)	232(3.68) 331(4.19)	221(3.84) 254(3.14) 341(4.28) 380(3.33)	221(3.82) 254(3.12) 340(4.28) 382(3.29)
4-Nitro-5-methoxy-2,1,3-benzoselenadiazole	340(3.72) 390(3.63)			
4-Nitro-5-methyl-2,1,3-benzoselenadiazole	338(4.27)			
4-Nitro-5-piperidyl-2,1,3-benzoselenadiazole	251(4.23) 264(3.98) 338(3.88) 445(4.01)			
4-Nitro-5-chloro-2,1,3-benzoselenadiazole	341(4.31) 430(2.73)			

TABLE 2

ULTRAVIOLET-VISIBLE ABSORPTION SPECTRA OF
2,1,3-BENZOTHIADIAZOLE AND
A DERIVATIVE

Compound	Values given as λmax in mμ (Log ε)		
	95% EtOH	50% H_2SO_4	95% H_2SO_4
2,1,3-Benzothiadiazole	222(4.16) 304(4.14) 310(4.14) 330(3.39)[2]	224(4.09) 314(4.17) 348(3.33)	223(4.08) 314(4.18) 360(3.23)
4-Nitro-2,1,3-benzothiadiazole	249(3.61) 314(4.11)		

TABLE 3

ULTRAVIOLET-VISIBLE ABSORPTION SPECTRA OF
TWO 8-SELENAPURINE COMPOUNDS
IN WATER

Compound	Values given as λmax in mμ (Log ε)
6-Amino-8-selenopurine	233(3.86) 339(3.93) 302(3.56) 388(3.19)
6-Hydroxy-8-selenopurine	236(3.89) 276(3.32) 337(4.00)

[2]Underlined wave lengths denote shoulders.

B. SYNTHESIS OF DERIVATIVES OF 2,1,3-BENZOSELENADIAZOLE[3]

1. 5-Chloro-2,1,3-benzoselenadiazole.—Twenty-one and one-half g. (0.15 mole) of 4-chloro-o-phenylenediamine was dissolved in a minimum amount of ethanol, at about 40°. To this solution was added 19.4 g. (0.15 mole) of selenous acid dissolved in a minimum amount of ethanol. The resulting solution was stirred for 15 minutes, cooled, and the precipitate collected. The yield was 16.5 g. Recrystallization twice from hexane gave a melting point of 118-119°.

Anal. Calcd. for $C_6H_3ClN_2Se$: N, 12.9. Found: N, 12.7.

2. 6-Chloro-4-nitro-2,1,3-benzoselenadiazole.—Ten g. (0.05 mole) of 5-chloro-3-nitro-o-phenylenediamine was dissolved in a minimum amount of ethanol at about 30°, and to this solution was added a solution of 9.03 g. (0.07 mole) of selenous acid also in a minimum amount of ethanol. The mixture was stirred for three minutes by hand, cooled, and filtered. The precipitate was washed with a little methanol. The yield was 6.7 g. After recrystallization from toluene, the melting point was 254°.

Anal. Calcd. for $C_6H_2ClN_3O_2Se$: N, 16.0. Found: N, 16.1.

3. 5-Methoxy-2,1,3-benzoselenadiazole.—Eight and four-tenths g. (0.05 mole) of 2-nitro-4-methoxyaniline was dissolved in 60 ml. of concentrated hydrochloric acid. To this solution was added a solution of 45.12 g. (0.2 mole) of stannous chloride in 60 ml. of concentrated

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[3]All melting points are uncorrected. Analyses were by Peninsular ChemResearch, Inc., Gainesville, Florida, and by Galbraith Laboratories, Knoxville, Tennessee.

hydrochloric acid. The resulting solution was reduced to half its volume and carefully neutralized with a concentrated solution of sodium hydroxide, keeping the temperature of the 4-methoxy-o-phenylenediamine solution below 30°. The resulting solution was extracted with three, 100 ml. portions of ether and the extracts reduced to a vol. of 100 ml. To this solution was added 8 g. (1.05 mole) of selenous acid dissolved in a minimum amount of ethanol-ether (1:1). The resulting reaction mixture was cooled and filtered, yielding 3.2 g. of product. Recrystallization from heptane gave a product melting at 110°-111°.

Anal. Calcd. for $C_7H_6N_2OSe$: N, 13.1. Found: N, 13.3.

4. 5-Ethoxy-2,1,3-benzoselenadiazole.—Four and four-tenths g. (0.025 mole) of 2-nitro-4-ethoxyaniline was dissolved in 30 ml. of concentrated hydrochloric acid. To this solution was added 22.6 g. (0.1 mole) of stannous chloride in 20 ml. of concentrated hydrochloric acid. After the initial reaction, the solution volume was reduced by one-half and cooled to 5° for two hours. The resulting precipitate was collected, dissolved in a minimum amount of water, neutralized with potassium acetate and filtered. To the filtrate, containing the free 4-ethoxy-o-phenylenediamine, was added 4.0 g. (0.032 mole) of selenous acid in 10 ml. of water. The reaction mixture was cooled and filtered to give 4.4 g. of product. The melting point when twice recrystallized from hexane was 99-100.5°. Hinsberg (98) reported a melting point of 103-104°.

5. 5-Methyl-2,1,3-benzoselenadiazole.—Six g. (0.04 mole) of

2-nitro-4-methylaniline was dissolved in 40 ml. of hot concentrated hydrochloric acid and 36.2 g. (0.16 mole) of stannous chloride, dissolved in 40 ml. of concentrated hydrochloric acid, was cautiously added. Following the initial reaction, the volume of the solution was reduced by one-half and let stand over night at about 5°. The resulting precipitate was collected, dissolved in a minimum amount of water, and neutralized with a concentrated sodium hydroxide solution, keeping the temperature at less than 30°. This mixture was filtered and the precipitate extracted with two, 100 ml. portions of ethanol, and the combined previous filtrate and these extracts were treated with 5.2 g. (0.04 mole) of selenous acid in 15 ml. of water. The reaction mixture was allowed to stand over night at about 5° and the resulting precipitate collected. The mother liquor was concentrated to one-half the original volume, and a second crop of material collected, weighing together 3.0 g. This material recrystallized once from pentane melted at 70-71°. Hinsberg (97) reports the melting point for this compound to be 72-73°.

6. 5-Dimethylamino-2,1,3-benzoselenadiazole.—This compound was obtained by starting with N,N-dimethylaniline and nitrating as directed by Romburgh (177) to obtain the N,N-dimethyl-3,4-dinitroaniline.

A solution of 114 g. (0.5 mole) of stannous chloride in 60 ml. of concentrated hydrochloric acid was added to 8.4 g. (0.04 mole) of N,N-dimethyl-3,4-dinitroaniline in 50 ml. of concentrated hydrochloric acid. This addition was carried out at temperatures less than 50° and

with mechanical stirring. The reaction mixture was then stirred at 50° for an additional two hours and allowed to stand over night at 5°. The resulting crystals were collected, dissolved in a minimum amount of water, and carefully neutralized with a concentrated sodium hydroxide solution, keeping the temperature below 20°. This mixture was filtered, and to the filtrate was immediately added 5.16 g. (0.04 mole) of selenous acid in 15 ml. of water. The reaction mixture was cooled and filtered, giving 2.0 g. of product. It melted at 107-109° after two recrystallizations from hexane.

Anal. Calcd. for $C_8H_9N_3Se$: N, 18.6. Found: N, 18.8.

7. 5-Nitro-2,1,3-benzoselenadiazole.—Thirteen and one-half g. (0.09 mole) of 4-nitro-o-phenylenediamine was dissolved in a minimum amount of ethanol and 11.6 g. (0.09 mole) of selenous acid in 20 ml. of ethanol was added. The mixture was stirred for 10 minutes, cooled, and filtered. The product yield was 13.0 g. and was purified from methyl-cellosolve, melting at 223-224°.

Anal. Calcd. for $C_6H_3N_3O_2Se$: N, 18.4. Found: N, 18.3.

8. 4,6-Dimethyl-2,1,3-benzoselenadiazole.—Ten and four-tenths g. (0.05 mole) of 2-nitro-4,6-dimethylacetanilide was dissolved in 20.0 ml. of methyl-cellosolve and to this solution was added 10.0 ml. of concentrated hydrochloric acid. This solution was refluxed for 1.5 hours and an equal volume of water added. This was cooled to 0° for 0.5 hour and the resulting precipitate collected, which was dissolved in 50.0 ml. of hot concentrated hydrochloric acid. A solution of 45.0 g. (0.2 mole) of stannous chloride in 40.0 ml. of concentrated

hydrochloric acid was cautiously added to this solution and the volume of the resulting solution was reduced by one-half and cooled. The resulting precipitate was dissolved in a minimum amount of water and the solution made distinctly alkaline with a concentrated solution of sodium hydroxide, the temperature being kept at less than 30°. The filtrate from this and two, 10 ml. extractions of the resulting precipitate were combined and 6.5 g. (0.05 mole) of selenous acid in 10 ml. of water was added to this solution of 4,6-dimethyl-o-phenylenediamine. The resulting reaction mixture was kept at 5° over night and the precipitate collected. The yield was 4.4 g. of the compound melting at 153-155° after two recrystallizations from hexane-heptane (1:1).

Anal. Calcd. for $C_8H_8N_2Se$: N, 13.3. Found: N, 13.2.

9. 5,6-Dichloro-2,1,3-benzoselenadiazole.—The 3 step synthesis involved in the preparation of this compound begins with the nitration of 3,4-dichloronitrobenzene as directed by Le Fevre (133).

One and two-tenths g. (0.005 mole) of 1,2-dinitro-4,5-dichlorobenzene was finely powdered and added to 10.0 g. (0.04 mole) of stannous chloride in 10 ml. of concentrated hydrochloric acid at 5°, with continuous efficient stirring. During this addition the temperature was kept below 50°. Then, the reaction mixture was stirred at 50° for an additional half hour, at which time, it was cooled to 5° and five ml. of concentrated hydrochloric acid added. The resulting precipitate was collected and washed with 10 ml. of concentrated hydrochloric acid. This precipitate was dissolved in a

minimum amount of water, made alkaline with solid potassium carbonate, and filtered. The filtrate and two, 10 ml. ethanol extracts of the precipitate were treated with 0.7 g. (0.005 mole) of selenous acid in 5 ml. of water. The mixture was cooled to 5° and the product collected. The yield was 1.0 g., melting at 162-163° when purified from hexane.

Anal. Calcd. for $C_6H_2Cl_2N_2Se$: N, 11.1. Found: N, 11.4.

10. 5-Amino-2,1,3-benzoselenadiazole.—Twenty-three g. (0.15 mole) of 4-nitro-o-phenylenediamine was suspended in 300 ml. of concentrated hydrochloric acid and to this was added a solution of 135.0 g. (0.55 mole) of stannous chloride in 150 ml. of concentrated hydrochloric acid. After the initial reaction, the volume of this solution was reduced by one-half and an equal volume of concentrated hydrochloric acid added. This was then cooled to 5° and the resulting precipitate collected and washed well with EtOH-HCl (1:1) and then washed with ether. This precipitate, 4-amino-o-phenylenediamine hydrochloride, was then dissolved in a minimum amount of water, and 15.0 g. (0.12 mole) of selenous acid dissolved in 20 ml. of water was added. The resulting mixture was made just basic with potassium acetate, stirred for 10 minutes and filtered. The yield was 12.7 g., which was purified by recrystallization from water. The compound melted at 147-148°. Hinsberg (98) reported 149-150° as the melting point of this compound.

11. 4-Amino-2,1,3-benzoselenadiazole.—Two and three-tenths g. (0.01 mole) of 4-nitro-2,1,3-benzoselenadiazole was added to a mixture

of 8.0 g. of zinc dust in 70 ml. of hot water. To this mixture was added 20.0 ml. of concentrated hydrochloric acid and the reaction mixture was refluxed for 30 minutes. This was then filtered hot, 50 ml. of concentrated hydrochloric acid being then added to the filtrate, and the mixture cooled to 5°. The resulting precipitate was collected (1.7 g. of 1,2,3-triaminobenzene dihydrochloride) and was washed with 20 ml. of 25% hydrochloric acid. This material was then dissolved in 2.0 ml. of water and neutralized with solid potassium acetate. One g. (0.008 mole) of selenous acid in 2 ml. of water was added at 0° and the reaction mixture stirred for 5 minutes and filtered. The yield was 1.78 g., melting at 159.5-160.5° after two recrystallizations from heptane.

Anal. Calcd. for $C_6H_5N_3Se$: N, 21.2. Found: N, 20.8.

12. Nitration of 2,1,3-benzoselenadiazole and its Derivatives.—The method reported here for the nitration of 2,1,3-benzoselenadiazole has been found to be applicable to certain of the substituted-2,1,3-benzoselenadiazole derivatives.

General Procedure.—To a solution 0.01 mole of 2,1,3-benzoselenadiazole (97), or one of its derivatives, in 4.0 ml. of concentrated sulfuric acid, at 0°, was added a solution of 1.0 ml. of HNO_3 (d 1.4) (0.015 mole) in 2 ml. of concentrated sulfuric acid. This solution, after standing for 30 minutes at room temperature, was poured over 10-20 g. of ice and the precipitate collected. Table 4 summarizes the results of this reaction with 2,1,3-benzoselenadiazole and its derivatives.

13. Reaction of 5-Methyl-4-nitro-2,1,3-benzoselenadiazole with Aromatic Aldehydes.—5-Methyl-4-nitro-2,1,3-benzoselenadiazole was found to react with aromatic aldehydes in the presence of a small amount of piperidine to give a series of substituted-stilbenes.

General Procedure.—A mixture of 0.015 mole of the aromatic aldehyde and 0.005 mole of 5-methyl-4-nitro-2,1,3-benzoselenadiazole was heated on an oil bath at 150° till homogeneous. Then, 2 drops of piperidine were added and the resulting reaction mixture heated for 30 minutes at 150°. The reaction products were cooled and mixed with a double volume of methanol and filtered.

The results of this reaction with various aldehydes are shown in Table 5.

14. Reaction of Amines with 5-Chloro-4-nitro-2,1,3-benzoselenadiazole.—5-Chloro-4-nitro-2,1,3-benzoselenadiazole was found to react with amines to give 4-nitro-5-substituted-amino-2,1,3-benzoselenadiazole compounds.

General Procedure.—To 0.15 mole of the amine at 100-110° was added 1.3 g. (0.05 mole) of finely divided 5-chloro-4-nitro-2,1,3-benzoselenadiazole. The extent of subsequent heating for each compound is shown in Table 6. The reaction mixture was cooled, filtered and washed well with methanol. The results of this reaction are listed in Table 6.

15. Acylation of 4- and 5-Amino-2,1,3-benzoselenadiazoles.—These compounds were, with two exceptions, prepared by the action of acid anhydrides on 4 and 5-amino-2,1,3-benzoselenadiazoles in a

benzene solvent.

General Procedure.—To a solution of 0.80 g. (0.004 mole) of the amino-2,1,3-benzoselenadiazole in 20-30 ml. of benzene was added 0.0045 mole of the acid anhydride. After 15-30 minutes of reflux, an approximately double volume of water was added and the reaction mixture heated to distill off the benzene. After the benzene had been removed in this manner, the remaining mixture was cooled to about 5° and the precipitate collected. The results of this reaction are listed in Table 7.

16. 4-Monofluoroacetylamino-2,1,3-benzoselenadiazole.—This compound was prepared by the reaction of 0.80 g. (0.004 mole) of 4-amino-2,1,3-benzoselenadiazole with 0.44 g. (0.0046 mole) of monofluoroacetyl chloride in a solution of 25 ml. of benzene and 0.8 ml. of pyridine. After a 15 minute reflux period, a double volume of water was added and the benzene was steam distilled off. Upon cooling and filtering the flask residue, the compound was isolated. The results of this reaction are contained in Table 7.

17. 4-Carbethoxyamino-2,1,3-benzoselenadiazole.—To 0.8 g. (0.004 mole) of 4-amino-2,1,3-benzoselenadiazole in 6 ml. of pyridine at 0° was added 0.44 ml. of ethyl chloroformate. The resulting solution was refluxed two minutes and cooled to precipitate the product. The results of this reaction are listed in Table 7.

18. 2,1,3-Benzothiadiazole.—To 15.0 g. (0.14 mole) of o-phenylenediamine in 240 ml. of benzene was added 45.0 g. (0.37 mole) of thionyl chloride. The reaction mixture was refluxed for 12 hours,

cooled, and filtered to give 17.6 g. of product. The compound was purified from pentane, melting at 42-44°. Efros and Levit (51) reported a melting point of 42-44° for this compound.

19. 4-Nitro-2,1,3-benzothiadiazole.—Nitration of 1.3 g. (0.01 mole) of this compound according to the method described for the nitration of 2,1,3-benzoselenadiazole, gave 1.7 g. of product melting at 105-106.5° after two recrystallizations from hexane. Efros and Levit (51) reported 107° as the melting point for this compound.

C. SYNTHESIS OF DERIVATIVES OF 8-SELENOPURINE

1. 4-Amino-6-hydroxy-2-mercaptopyrimidine.—This compound was prepared according to the method of Traube (210). Thus, 48.0 g. (0.6 mole) of thiourea was condensed with 66.0 g. (0.5 mole) of ethyl cyanoacetate in a sodium ethoxide solution to give 58.0 g. of product, isolated by acidification of the diluted reaction mixture with acetic acid.

2. 4,5-Diamino-6-hydroxy-2-mercaptopyrimidine.—This compound was prepared by a known procedure (55, 210). Twenty g. (0.18 mole) of 4-amino-6-hydroxy-2-mercaptopyrimidine was nitrosated using 10.0 g. of sodium nitrite to give the corresponding 5-nitroso-compound. This was collected and used directly in reduction by sodium hydrosulfite to give 21 g. of the amorphous diamine.

3. 4,5-Diamino-6-hydroxypyrimidine.—Preparation of this compound was carried out as described by Elion et al. (55). To a solution of 0.9 g. of sodium carbonate in 50 ml. of water was added 2.5 g.

(0.02 mole) of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine. After the material had dissolved, 7.0 g. of Raney nickel catalyst was added and the mixture refluxed for two hours. The filtrate from this reaction was neutralized with concentrated hydrochloric acid and used directly in the next reaction.

4. 6-Hydroxy-8-selenopurine.—To the previously described solution of 4,5-diamino-6-hydroxypyrimidine was added a solution of 2.0 g. (0.02 mole) of selenous acid in 10 ml. of water. After standing 12 hours at room temperature, long light yellow needles were deposited, weighing 1.9 g. An analytical sample was prepared by two recrystallizations from water, melting higher than 360°.

Anal. Calcd. for $C_4H_2N_4OSe$: N, 27.9. Found: N, 28.4.

5. 4,6-Diamino-2-mercaptopyrimidine.—This compound was prepared by the method of Hoffer (105). Sixty-six g. (1.0 mole) of malononitrile and 76.0 g. (1.2 mole) of thiourea were condensed in an anhydrous sodium ethoxide solution to give 110.0 g. of product, upon acidification of the diluted reaction mixture.

6. 4,6-Diaminopyrimidine.—The preparation of this compound was carried out according to the method of Brown (25). Forty g. of Raney nickel catalyst was added to a solution of 3 liters of water containing 80 ml. of concentrated ammonium hydroxide and 20 g. (0.14 mole) of 4,6-diamino-2-mercaptopyrimidine. This mixture was refluxed 1 hour, filtered, and evaporated to dryness to give 12.0 g. of product. Purification from water gave a compound melting at 270-271°. Brown (25) reported a melting point of 268-270°.

7. 5-Nitro-4,6-diaminopyrimidine.—The procedure of Brown (25) was used for the synthesis of this compound. Five g. (0.05 mole) of 4,6-diaminopyrimidine was nitrated in 10 ml. of sulfuric acid using 8 ml. of yellow fuming nitric acid (d 1.5). The mixture, after being poured over ice, was neutralized and filtered to give the desired product. Drying the compound was avoided, since it was found that the following reduction proceeded much better by using the nitro-compound directly.

8. 4,5,6-Triaminopyrimidine.—The 4,6-diamino-5-nitropyrimidine isolated from the previous reaction mixture was reduced according to the method of Evans et al. (58). Thus, the wet nitro-compound and 8.4 g. of hydrogen reduced iron powder were suspended in 320 ml. of water containing 0.4 ml. of concentrated hydrochloric acid. After heating 2.5 hours on a boiling water bath, the reaction mixture was filtered and cooled. This solution, containing the 4,5,6-triaminopyrimidine was used in the following reaction.

9. 6-Amino-8-selenopurine.—To the above reaction filtrate was added 5.4 g. (0.05 mole) of selenous acid in 20 ml. of water. After standing 24 hours, 4.0 g. of product was collected. The compound was recrystallized from water and did not melt below 360°.

Anal. Calcd. for $C_4H_3N_5Se$: N, 35.0. Found: N, 35.3.

10. 4,6-Pyrimidinediol.—The procedure of Hull (114) was modified by the use of an excess of ethyl formate for the preparation of this compound. To a solution of 48.0 g. of sodium in 1500 ml. of absolute ethanol was added 222.0 g. of ethyl formate and 102.0 g.

(1.0 mole) of finely ground malonamide. The resulting reaction mixture was stirred well for 2 hours at a temperature sufficiently high to maintain a gentle reflux. The reaction mixture was let stand over night at room temperature, was filtered, and washed with 100 ml. of cold absolute ethanol. The resulting precipitate was dissolved in 500 ml. of water and made acid with 6 N hydrochloric acid. The resulting precipitate was then collected and dried over phosphoric anhydride in a vacuum desiccator. The yield was 62.0 g. of product melting at greater than 360°.

11. 5-Nitro-4,6-pyrimidinediol.—This compound was obtained according to the procedure of Boon et al. (23). To a mixture of 20.4 g. of yellow fuming nitric acid (d 1.5) and 36.0 g. of acetic acid was added 11.2 g. (0.1 mole) of 4,6-pyrimidinediol. The mixture was stirred ten minutes, while the temperature was raised to 35°. At this time, 3 ml. of yellow fuming nitric acid was added. The exothermic reaction mixture was immediately poured into 100 g. of crushed ice. After standing several hours, the product was collected, weighing 11.0 g.

12. 4,6-Dichloro-5-nitropyrimidine.—Following the directions of Boon et al. (23), 93.0 g. (0.77 mole) of dimethylaniline was added to a suspension of 78.5 g. (0.5 mole) of 5-nitro-4,6-pyrimidinediol in 505 g. (3.3 mole) of phosphorous oxychloride. This reaction mixture was heated on an oil bath at 125-130° for 1 hour, after which the excess phosphorous oxychloride was distilled off at water aspirator pressure. The flask residue was poured over 800 g. of ice and

filtered. The resulting filtrate was extracted with three, 300 ml. portions of ether, each extract being used to extract the filter cake. The combined extracts were washed with water, dried, and the ether removed to give 80.0 g. of product melting at 103-104° from hexane.

13. 4-Amino-5-nitro-6-chloropyrimidine.—The method of Boon et al. (23) was used for the preparation of this compound. To 38.8 g. (0.2 mole) of 4,6-dichloro-5-nitropyrimidine dissolved in 300 ml. of ether was added 30.0 ml. of methanol containing 6.9 g. (0.4 mole) of ammonia. The resulting precipitate was extracted with two, 100 ml. portions of ethyl acetate. The above filtrate and extracts were evaporated to dryness and extracted with hexane. This residue recrystallized from benzene gave 15.0 g. of product melting at 153-155°. Boon et al. reported 155-156° as the melting point of this compound.

14. 6-Chloro-4,5-diaminopyrimidine.—Robins (172) has reported the preparation of this compound. Thus, 0.5 g. (0.03 mole) of 4-amino-6-chloro-5-nitropyrimidine was cautiously added to a mixture of 3.0 g. of zinc dust in 15 ml. of water. This reaction mixture, after heating at 100° for 10 minutes, was filtered and the filtrate brought to pH = 10 with concentrated ammonium hydroxide. Upon cooling, 0.2 g. of light needles were obtained, melting at 252°. Robins (172) recorded a melting point of 252°.

15. 4-Amino-6-morpholyl-5-nitropyrimidine.—Following the directions of Daly and Christensen (43), 3.6 g. (0.02 mole) of 4-amino-6-chloro-5-nitropyrimidine was added to 72 ml. of n-butanol

containing 7.3 g. (0.08 mole) of morpholine. After refluxing for 1.5 hours the resulting solution was cooled to give 2.0 g. of product melting at 176-179°. Daly and Christensen (43) reported this same melting point.

16. 6-Morpholyl-4,5-diaminopyrimidine.—The above authors (43) described the preparation of this compound. Thus, 1.0 g. (0.004 mole) of 4-amino-6-morpholyl-5-nitropyrimidine was suspended in 75.0 ml. of methanol containing 2.0 g. of Raney nickel catalyst. This mixture was shaken under 45 lbs. hydrogen pressure for 2 hours. After filtering, the methanol solution was used directly in the next reaction.

17. 6-Morpholyl-8-selenopurine.—To the above solution of 4,5-diamino-6-morpholylpyrimidine was added a solution of 0.5 g. (0.004 mole) of selenous acid in 10 ml. of methanol. After standing for 3 days, large yellow crystals of product were deposited, weighing 0.7 g. The compound melted at 205.5-206°.

Anal. Calcd. for $C_8H_9N_5OSe$: N, 25.9. Found: N, 25.5.

18. 4,6-Diamino-2-hydroxypyrimidine.—Following the procedure of Bendich et al. (14), 10.8 g. (0.076 mole) of 4,6-diamino-2-mercaptopyrimidine and 11.0 g. (0.1 mole) of chloroacetic acid were added to 150 ml. of water and the resulting solution refluxed 1 hour. After carefully adding 19.0 ml. of 18 N sulfuric acid, the mixture was refluxed 1 more hour and cooled to give 7.9 g. of 4,6-diamino-2-hydroxypyrimidine sulfate.

19. 2-Hydroxy-4,5,6-triaminopyrimidine.—This compound was synthesized by the method of Bendich et al. (14). Five g.

(0.022 mole) of 4,6-diamino-2-hydroxypyrimidine sulfate was dissolved in 670 ml. of boiling water and treated with 3.0 g. (0.044 mole) of sodium nitrite in 50 ml. of water. After 5 minutes, an equal volume of crushed ice was added to the reaction mixture and the 5-nitroso-compound was collected. This was immediately suspended in 180 ml. of water, and 10.0 g. of sodium hydrosulfite was added. The mixture was boiled 3 minutes and 23 ml. of 18 N sulfuric acid was cautiously added. Cooling gave 3.9 g. of 2-hydroxy-4,5,6-triaminopyrimidine sulfate as white needles.

TABLE 4

NITRATION OF 2,1,3-BENZOSELENADIAZOLE AND ITS DERIVATIVES

Substituted-2,1,3-benzoselenadiazole	Product	Yield, grams	Purification Solvent	M.p. C°	Nitrogen, % Calc.	Found
None	4-Nitro-2,1,3-benzoselenadiazole	2.3	Ethanol	219-221	18.4	18.4
5-Chloro-	5-Chloro-4-nitro-2,1,3-benzoselenadiazole	2.2	Methyl-Cellosolve	228-229	16.0	15.0
5-Methyl-	5-Methyl-4-nitro-2,1,3-benzoselenadiazole	1.5	Xylene	192-194	17.7	17.2
5-Methoxy-	5-Methoxy-4-nitro-2,1,3-benzoselenadiazole	1.8	Xylene	234-236	16.3	16.3
5-Ethoxy-	5-Ethoxy-4-nitro-2,1,3-benzoselenadiazole	1.9	Xylene	196-199	15.4	15.6
4,6-Dimethyl-	4,6-Dimethyl-7-nitro-2,1,3-benzoselenadiazole	1.8	Heptane	163-165	16.4	16.0
5,6-Dichloro-	5,6-Dichloro-4-nitro-2,1,3-benzoselenadiazole	2.8	Benzene-Heptane(1:1)	222-224	14.1	14.2

TABLE 5

REACTION OF 5-METHYL-4-NITRO-2,1,3-BENZOSELENADIAZOLE WITH AROMATIC ALDEHYDES

Aldehyde	Product	Yield, grams	Purification Solvent	M.p. C°	Nitrogen, % Calc.	Found
p-Chloro-benzaldehyde	5-(p-Chlorostyryl)-4-nitro-2,1,3-benzoselenadiazole	1.1	Methyl-Cellosolve	239-241	11.5	9.6
1-Naphthaldehyde	4-Nitro-5-benzo-a-styryl-2,1,3-benzoselenadiazole	0.9	Toluene	251-253	11.0	10.8
3,4-Dimethoxy-benzaldehyde	5-(3,4-Dimethoxystyryl)-4-nitro-2,1,3-benzoselenadiazole	1.0	Methyl-Cellosolve	249-251	10.7	10.8
p-Methyl-benzaldehyde	5-(p-Methylstyryl)-4-nitro-2,1,3-benzoselenadiazole	1.3	Xylene	248-250	12.2	12.0
p-Dimethylamino-benzaldehyde	5-(p-Dimethylamino-styryl)-4-nitro-2,1,3-benzoselenadiazole	1.5	Methyl-Cellosolve	246-247	15.0	14.7
Benzaldehyde	4-Nitro-5-styryl-2,1,3-benzoselenadiazole	1.0	Benzene	231-233	12.7	12.4

TABLE 6

REACTION OF AMINES WITH 5-CHLORO-4-NITRO-2,1,3-BENZOSELENADIAZOLE

Amine	Added Heating	Product	Yield, grams	Purification Solvent	M.p. C°	Nitrogen, % Calc.	Found
Aniline	None	5-Anilino-4-nitro-2,1,3-benzoselenadiazole	1.3	Methyl-Cellosolve	254	17.6	17.7
Piperidine	Reflux, 5 min.	4-Nitro-5-piperidyl-2,1,3-benzoselenadiazole	0.9	Heptane	138-139	18.0	17.9
N-Phenyl-piperazine	100°, 15 min.	4-Nitro-5-(N-phenylpiperazyl)-2,1,3-benzoselenadiazole	1.5	Xylene	203-204	18.0	18.2
Morpholine	Reflux, 15 min.	5-Morpholyl-4-nitro-2,1,3-benzoselenadiazole	0.8	Heptane	157-159	17.9	17.9

TABLE 7

DERIVATIVES OF 4- AND 5-AMINO-2,1,3-BENZOSELENADIAZOLES

Compound	Reagent	Product	Yield, grams	Purification Solvent	M.p. C°	Nitrogen, % Calc.	Found
A. 4-Amino-derivatives							
	Acetic anhydride	4-Acetylamino-2,1,3-benzoselenadiazole	0.75	Heptane	177-178	17.5	17.6
	Mono-fluoro-acetyl chloride	4-Monofluoroacetyl-amino-2,1,3-benzo-selenadiazole	0.85	Hexane	135-136	16.3	16.4
	Difluoro-acetic anhydride	4-Difluoroacetyl-amino-2,1,3-benzo-selenadiazole	0.80	Hexane	145-146	15.0	15.2
	Trifluoro-acetic anhydride	4-Trifluoroacetyl-amino-2,1,3-benzo-selenadiazole	1.1	Hexane	140-141	14.3	14.4
	Perfluoropro-pionic anhy-dride	4-Perfluoropropion-ylamino-2,1,3-benzo selenadiazole	1.1	Heptane	152	12.2	12.0

TABLE 7—Continued

Compound	Reagent	Product	Yield, grams	Purification Solvent	M.p. C°	Nitrogen, % Calc.	Found
	Perfluorobutyric anhydride	4-Perfluorobutyryl-amino-2,1,3-benzoselenadiazole	1.3	Hexane	129-131	10.5	10.7
	Ethyl chloroformate	4-Carbethoxyamino-2,1,3-benzoselenadiazole	0.2	Pentane	89-90	15.5	15.7
B. 5-Amino-derivatives							
	Difluoro-acetic anhydride	5-Difluoroacetyl-amino-2,1,3-benzoselenadiazole	1.2	Heptane	194-195	15.2	15.0
	Perfluoropropionic anhydride	5-Perfluoropropionylamino-2,1,3-benzoselenadiazole	0.5	Benzene	224-226	15.2	15.3

D. RESULTS OF SCREENING TESTS AGAINST SARCOMA 180

Some of the compounds resulting from this investigation have been tested for anti-cancer action by the Sloan-Kettering Institute for Cancer Research. The method used in this screening program has been discussed by Clarke (260).

Five female Swiss albino mice, weighing from eighteen to twenty-two grams and bearing uniformly cut five milligram pieces of Sarcoma 180 implanted subcutaneously in the right axillary region, were used. Daily therapy was initiated twenty-four hours after implantation and continued for seven successive days. The agent tested was injected into the peritoneal cavity, the full daily dose being divided into two equal portions and injected about seven hours apart. The day following the last injection, the mice were weighed and changes in weight of each surviving animal noted and averaged. Tumor diameters were measured through the skin to the nearest tenth of a millimeter. The largest and smallest diameters of the tumor were averaged and considered as an "average tumor diameter."

Control animals with the same tumor were treated the same except for the absence of the compound injection. The average tumor diameter of the experimental animals was compared with that of these controls. An experimental average tumor diameter 75-100% of the control value was given a (-) rating. Compounds which inhibit growth of the tumor so that the average tumor diameter was 25-75% of the control value were given a ($\pm$) or borderline rating. Marked inhibition of tumor growth so that the average tumor diameter of

experimental animals was 0-25% of controls was given a (+) rating. A (?) rating was given those compounds which in testing were too toxic to establish a rating.

From the results given in Tables 8 and 9, it can be seen that none of the compounds were active in this test system.

The results do show, however, that the toxicity factor due to the presence of selenium is not critical. Dose levels of 500 mg./kg. as found in 4-amino-2,1,3-benzoselenadiazole can be classified as relatively non-toxic.

Although all of these compounds were found inactive in this test system, it should be kept in mind that cancer is really a group of diseases. Agents inactive against one form of cancer may be active toward another form. Hence, these results against a sarcomateous growth should not be considered final.

TABLE 8

RESULTS OF SARCOMA 180 INHIBITION TESTS
ON 2,1,3-BENZOSELENADIAZOLE AND
ITS DERIVATIVES

Substituent	Dose, mg./kg.	Result on Sarc. 180	Change in weight[4]	Deaths
None-	8	-	-0.5/-1.0	0
	30	?		4
	125	?		5
4-Nitro-	30	-	+0.5/+2.0	0
	60	?		4
	125	?		5
4-Amino-	125	-	-0.5/0.0	0
	500	-	0.0/+0.5	0
6-Chloro-4-nitro-	125	-	-2.5/-1.0	0
	250	?		5
	500	?		5
5-Methoxy-	30	-	-3.0/-0.5	1
	60	?		4
	125	?		4
5-Chloro-	30	-	-1.5/+1.0	1
	60	?		4
	125	?		5
5-Nitro-	125	-	+1.0/-1.0	2
	250	-	+0.5/+2.0	0
	500	?		5
5-Amino-	30	-	-4.5/-0.5	1
	125	?		4

[4]Average change in test animal/average change in controls.

TABLE 8—Continued

Substituent	Dose, mg./kg.	Result on Sarc. 180	Change in weight	Deaths
4,6-Dimethyl-	30	-	-1.5/+2.0	0
	60	-	-1.0/+0.5	2
	125	?		3
	500	?		5
5-Methyl-	30	-	-2.0/+1.0	0
	60	?		4
	100	?		3

TABLE 9

RESULTS OF SARCOMA 180 INHIBITION TESTS
ON TWO 8-SELENOPURINE DERIVATIVES

Substituent	Dose, mg./kg.	Result on Sarc. 180	Change in weight	Deaths
6-Amino-	125	-	+2.5/+1.0	0
	250	?		4
	500	?		2
6-Hydroxy-	30	-	+2.0/+1.5	0
	60	-	-3.5/+0.5	0
	125	?		3

V. SUMMARY

A. Since derivatives of benzimidazole and purine have been found to possess antitumor activity, analogs of these compounds were chosen as possible cancer chemotherapeutic agents. These analogs all possess a selenium atom in that position corresponding to the 6-position of the purine nucleus.

B. The reaction of aromatic ortho-diamines with selenous acid was used to prepare these compounds. Thus, substituted-ortho-phenylenediamines in reaction with selenous acid gave a series of 2,1,3-benzoselenadiazole compounds. Substituted-4,5-diaminopyrimidines, in a similar reaction, gave a series of substituted-8-selenopurines. The ultraviolet-visible absorption spectra of some of these compounds were determined and discussed.

C. Sixty-one compounds were synthesized. Of these, thirty-seven are new. Twelve of these compounds have been submitted to the Sloan-Kettering Institute for Cancer Research for testing. The ultraviolet-visible absorption spectra and structure of these compounds were compared with those of 2,1,3-benzothiadiazole and purine compounds.

D. The following compounds were submitted for testing for anti-cancer activity: 2,1,3-benzoselenadiazole, 4-nitro-2,1,3-benzoselenadiazole, 4-amino-2,1,3-benzoselenadiazole, 6-chloro-4-nitro-2,1,3-benzoselenadiazole, 4,6-dimethyl-2,1,3-benzoselenadiazole,

5-methoxy-2,1,3-benzoselenadiazole, 5-chloro-2,1,3-benzoselenadiazole, 5-nitro-2,1,3-benzoselenadiazole, 5-amino-2,1,3-benzoselenadiazole, 5-methyl-2,1,3-benzoselenadiazole, 6-hydroxy-8-selenopurine, and 6-amino-8-selenopurine. None of these compounds showed any activity in this preliminary Sarcoma 180 screening. There are many forms of cancer. Agents active against one form may not be active against another. For this reason, the compounds tested here should be tested further in other systems.

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